Fig. 1.

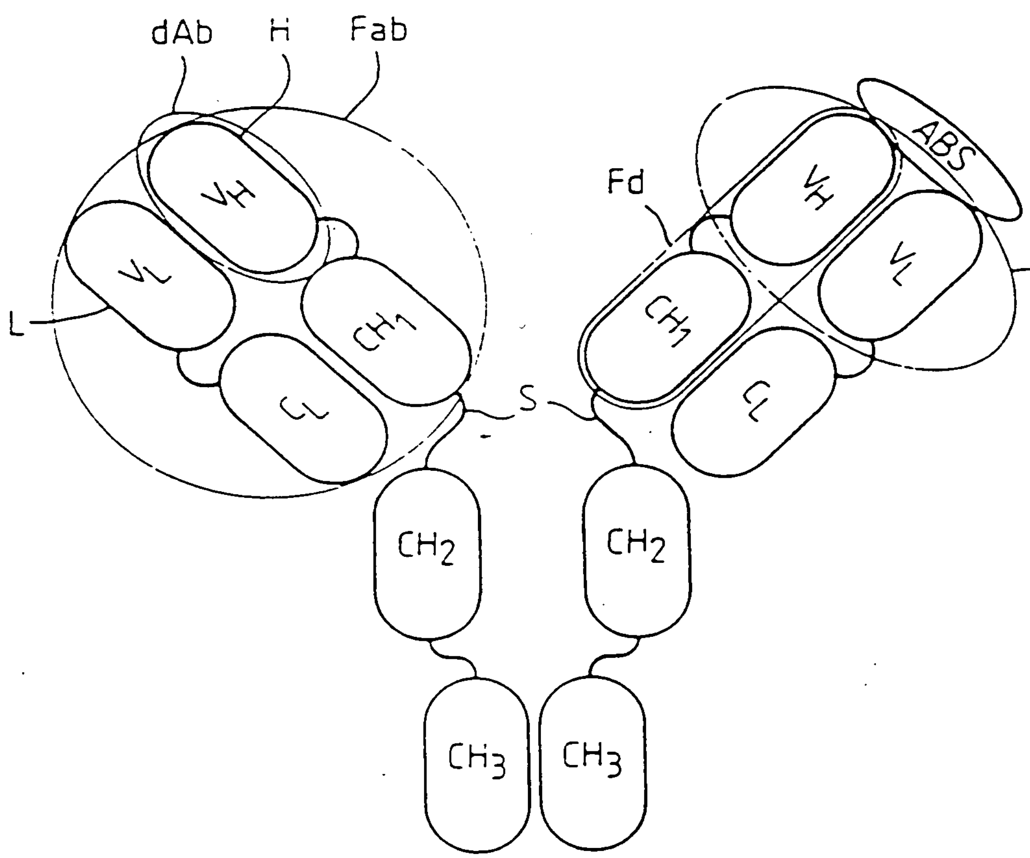

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Fig. 2 (i)

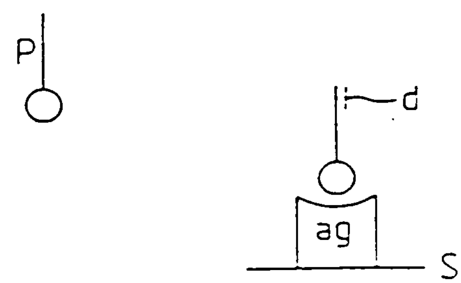
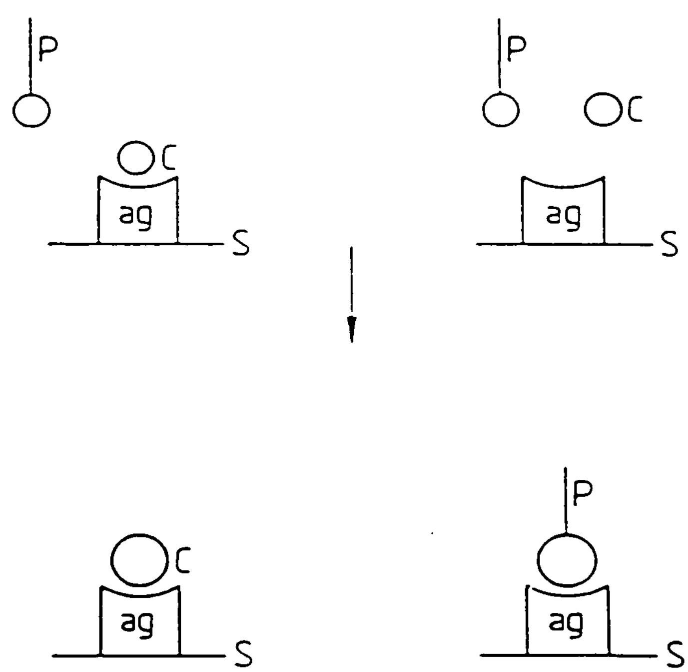


Fig. 2(ii)



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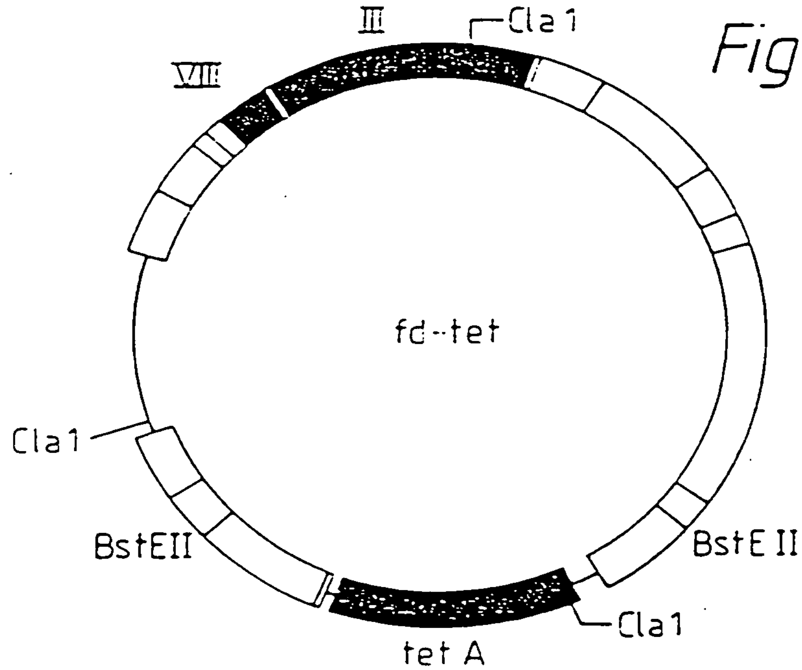

Fig.3.

fd - tet

cleave with BstEII

fill in with Klenow

re-ligate

FDT δ Bst

in vitro mutagenesis (oligo 1)

FDTPs/Bs

in vitro mutagenesis (oligo 2)

FDTPs/Xh

Fig. 4.1

(1653)
Oligo 1 ACA ACT TTC AAC AGT TGA GGA GAC GGT GAC CGT AAG CTT CTG CAG TTG GAC CTG AGC
GGA GTG AGA ATA (1620)

(1653)
Oligo 2 ACA ACT TTC AAC AGT TTC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG

(1704)
Oligo 3 GTC GTC TTT CCA GAC GTT AGT

Fig. 4.2

GENE III GENE III

SIGNAL
CLEAVAGE SITE

(1624) (1650)
A TCT CAC TCC GCT GAA ACT GTT GAA AGT

Q V Q L Q V T V S S
B TCT CAC TCC GCT CAG GTC CAA CTG CAG AAG CTT ACG GTC ACC GTC TCC TCA ACT GTT GAA AGT
PstI BstEII

Q V Q L Q L E I K R
C TCT CAC TCC GCT CAG GTC CAA CTG CAG GAG CTC GAG ATC AAA CGG GAA ACT GTT GAA AGT
PstI XhoI

Fig. 5.

```
                          rbs               M  K  Y  L  L  P  T  A  A
GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC
        10        20        30        40        50        60
SphI
        PelB leader
A  G  L  L  L  L  A  A  Q  P  A  M  A  Q  V  Q  L  Q  E  S
GCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAGGAGTCA
        70        80        90       100       110       120
                                                   PstI

G  P  G  L  V  A  P  S  Q  S  L  S  I  T  C  T  V  S  G  F
GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACCGTCTCAGGGTTC
       130       140       150       160       170       180

S  L  T  G  Y  G  V  N  W  V  R  Q  P  P  G  K  G  L  E  W
TCATTAACCGGCTATGGTGTAAACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG
       190       200       210       220       230       240

                              VHD1.3
L  G  M  I  W  G  D  G  N  T  D  Y  N  S  A  L  K  S  R  L
CTGGGAATGATTTGGGGTGATGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTG
       250       260       270       280       290       300

S  I  S  K  D  N  S  K  S  Q  V  F  L  K  M  N  S  L  H  T
AGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT
       310       320       330       340       350       360

D  D  T  A  R  Y  Y  C  A  R  E  R  D  Y  R  L  D  Y  W  G
GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC
       370       380       390       400       410       420

                                       Linker Peptide
Q  G  T  T  V  T  V  S  S  G  G  G  G  S  G  G  G  G  S  G
CAAGGCACCACGGTCACCGTCTCCTCAggtggaggcggttcaggcggaggtggctctggc
       430       440       450       460       470       480
          BstEII

G  G  G  S  D  I  E  L  T  Q  S  P  A  S  L  S  A  S  V  G
ggtggcggatcgGACATCGAGCTCACTCAGTCTCCAGCCTCCCTTTCTGCGTCTGTGGGA
       490       500       510       520       530       540
                SacI
```

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Fig. 5 cont.

```
 E  T  V  T  I  T  C  R  A  S  G  N  I  H  N  Y  L  A  W  Y
GAAACTGTCACCATCACATGTCGAGCAAGTGGGAATATTCACAATTATTTAGCATGGTAT
        550       560       570       580       590       600

 Q  Q  K  Q  G  K  S  P  Q  L  L  V  Y  Y  T  T  T  L  A  D
CAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATTATACAACAACCTTAGCAGAT
        610       620       630       640       650       660

                          VKD1.3
 G  V  P  S  R  F  S  G  S  G  S  G  T  Q  Y  S  L  K  I  N
GGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGAACACAATATTCTCTCAAGATCAAC
        670       680       690       700       710       720

 S  L  Q  P  E  D  F  G  S  Y  Y  C  Q  H  F  W  S  T  P  R
AGCCTGCAACCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTTGGAGTACTCCTCGG
        730       740       750       760       770       780

                                      Myc Tag (TAG1)
 T  F  G  G  G  T  K  L  E  I  K  R  E  Q  K  L  I  S  E  E
ACGTTCGGTGGAGGGACCAAGCTCGAGATCAAACGGGAACAAAAACTCATCTCAGAAGAG
        790       800       810       820       830       840
                      XhoI

 D  L  N  *  *
GATCTGAATTAATAATGATCAAACGGTAATAAGGATCCAGCTCGAATTC
        850       860       870       880
                                          EcoRI
```

Fig. 6.

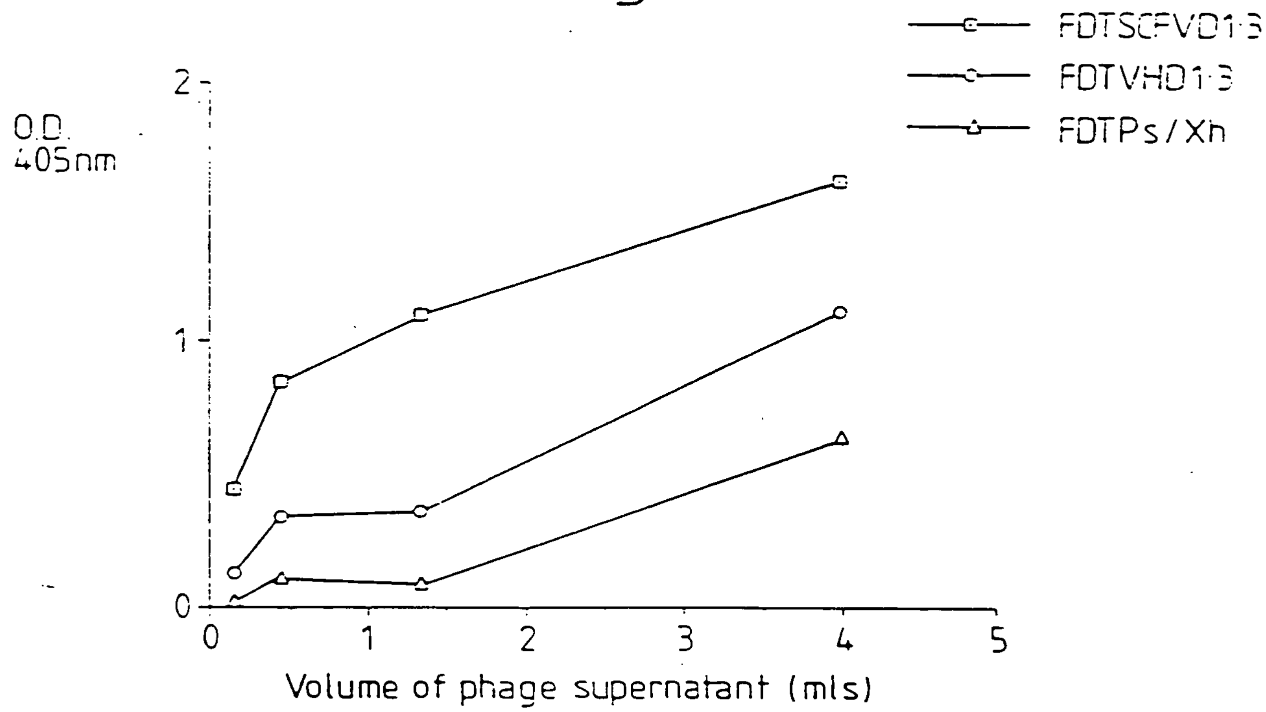
FDTSCFVD1·3
FDTVHD1·3
FDTPs/Xh
O.D. 405nm
2
1
0
0 1 2 3 4 5
Volume of phage supernatant (mls)

Fig. 7.

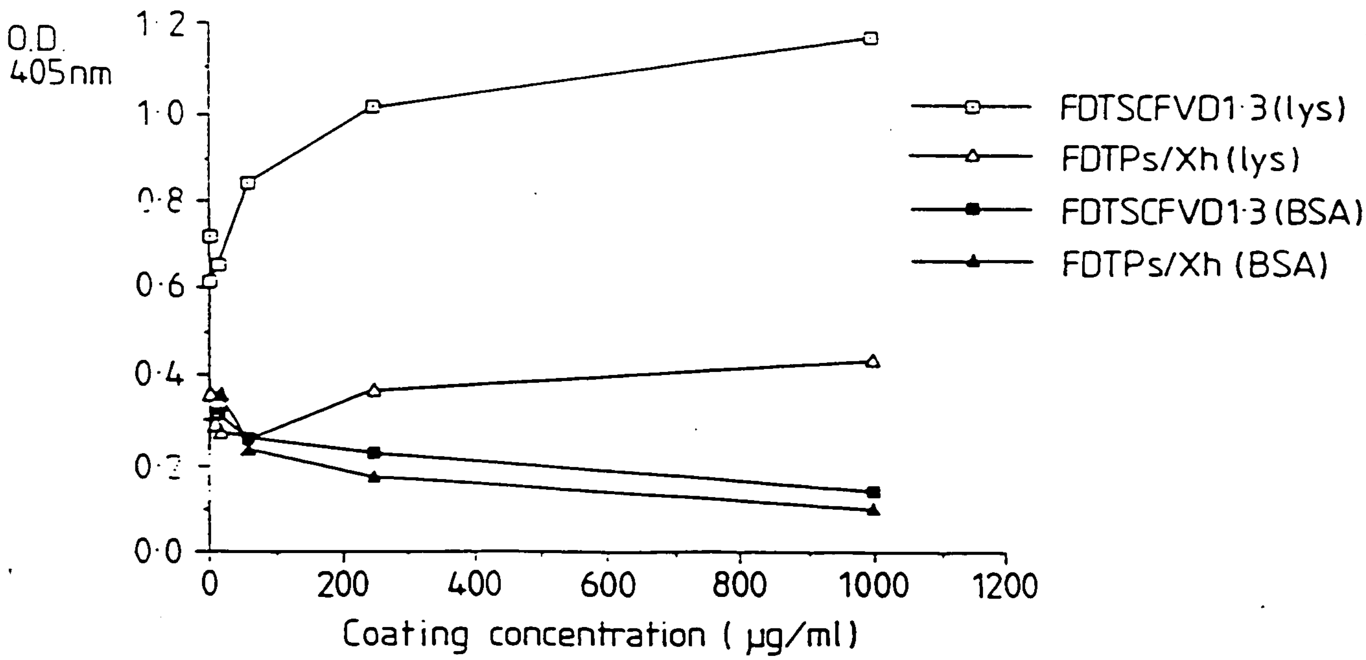
O.D. 405nm
1·2
1·0
0·8
0·6
0·4
0·2
0·0
0 200 400 600 800 1000 1200
Coating concentration (μg/ml)
FDTSCFVD1·3 (lys)
FDTPs/Xh (lys)
FDTSCFVD1·3 (BSA)
FDTPs/Xh (BSA)

Fig. 8.

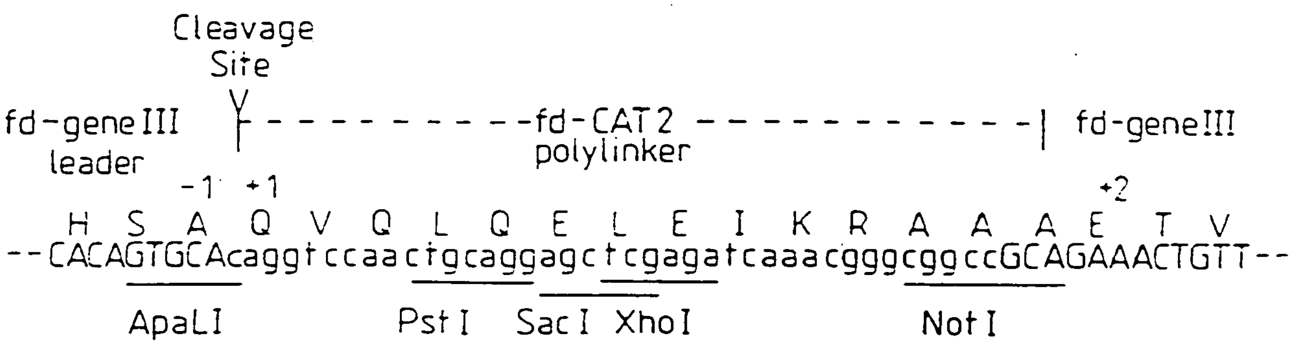
Cleavage Site
fd-gene III leader
fd-CAT2 polylinker
fd-gene III
-1 +1
+2
H S A Q V Q L Q E L E I K R A A A E T V
--CACAGTGCAcaggtccaactgcaggagctcgagatcaaacgggcggccGCAGAAACTGTT--
ApaLI
Pst I
Sac I
Xho I
Not I

Fig. 9.

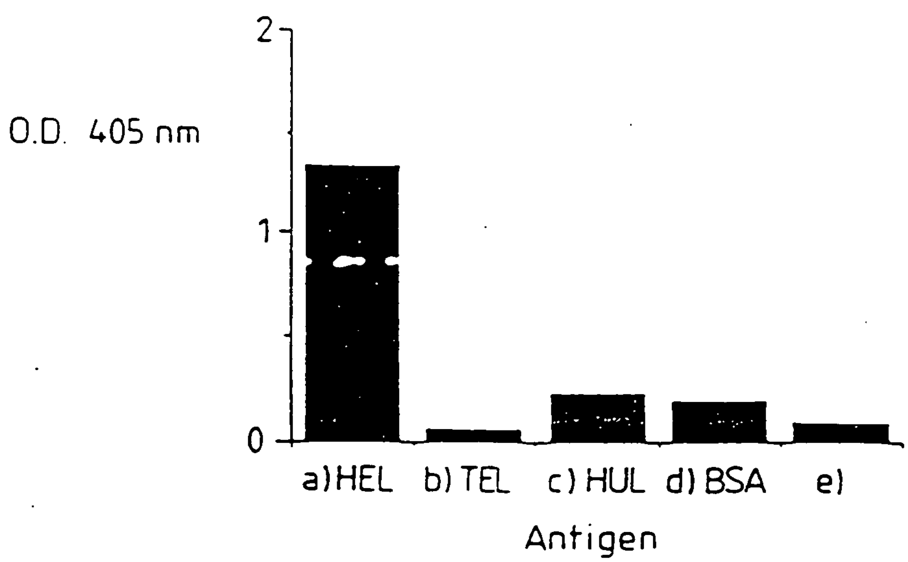
O.D. 405 nm
2
1
0
a) HEL
b) TEL
c) HUL
d) BSA
e)
Antigen

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Fig.10.

```
                                  M  K  Y  L  L  P  T  A  A
GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC
        10        20        30        40        50        60

 A  G  L  L  L  L  A  A  Q  P  A  M  A  Q  V  Q  L  Q  E  S
GCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAGGAGTCA
        70        80        90       100       110       120

 G  P  G  L  V  A  P  S  Q  S  L  S  I  T  C  T  V  S  G  F
GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACCGTCTCAGGGTTC
       130       140       150       160       170       180

 S  L  T  G  Y  G  V  N  W  V  R  Q  P  P  G  K  G  L  E  W
TCATTAACCGGCTATGGTGTAAACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG
       190       200       210       220       230       240

 L  G  M  I  W  G  D  G  N  T  D  Y  N  S  A  L  K  S  R  L
CTGGGAATGATTTGGGGTGATGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTG
       250       260       270       280       290       300

 S  I  S  K  D  N  S  K  S  Q  V  F  L  K  M  N  S  L  H  T
AGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT
       310       320       330       340       350       360

 D  D  T  A  R  Y  Y  C  A  R  E  R  D  Y  R  L  D  Y  W  G
GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC
       370       380       390       400       410       420

 Q  G  T  T  V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L
CAAGGCACCACGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG
       430       440       450       460       470       480

 A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D
GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
       490       500       510       520       530       540
```

Fig.10 cont.(1)

```
Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC
       550       560       570       580       590       600

T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V
ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG
       610       620       630       640       650       660

P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N
CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC
       670       680       690       700       710       720

T  K  V  D  K  K  V  E  P  K  S  S  *  *
ACCAAGGTCGACAAGAAAGTTGAGCCCAAATCTTCATAATAACCCGGGAGCTTGCATGCA
       730       740       750       760       770       780.

                           M  K  Y  L  L  P  T  A  A  A  G  L
AATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCCGCTGGAT
       790       800       810       820       830       840

  L  L  L  A  A  Q  P  A  M  A  D  I  E  L  T  Q  S  P  A  S
TGTTATTACTCGCTGCCCAACCAGCGATGGCCGACATCGAGCTCACCCAGTCTCCAGCCT
       850       860       870       880       890       900

  L  S  A  S  V  G  E  T  V  T  I  T  C  R  A  S  G  N  I  H
CCCTTTCTGCGTCTGTGGGAGAAACTGTCACCATCACATGTCGAGCAAGTGGGAATATTC
       910       920       930       940       950       960

  N  Y  L  A  W  Y  Q  Q  K  Q  G  K  S  P  Q  L  L  V  Y  Y
ACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATT
       970       980       990      1000      1010      1020
```

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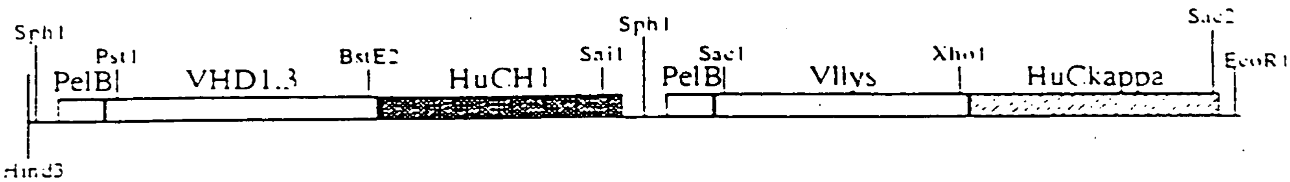
Fig.10 cont.(2)

```
   T  T  T  L  A  D  G  V  P  S  R  F  S  G  S  G  S  G  T  Q
ATACAACAACCTTAGCAGATGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGAACAC
       1030      1040      1050      1060      1070      1080

   Y  S  L  K  I  N  S  L  Q  P  E  D  F  G  S  Y  Y  C  Q  H
AATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACTGTCAAC
       1090      1100      1110      1120      1130      1140

   F  W  S  T  P  R  T  F  G  G  G  T  K  L  E  I  K  R  T  V
ATTTTTGGAGTACTCCTCGGACGTTCGGTGGAGGCACCAAGCTCGAGATCAAACGGACTG
       1150      1160      1170      1180      1190      1200

   A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A
TGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG
       1210      1220      1230      1240      1250      1260

   S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q  W  K  V
CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGG
       1270      1280      1290      1300      1310      1320

   D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D
TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGG
       1330      1340      1350      1360      1370      1380

   S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K
ACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACA
       1390      1400      1410      1420      1430      1440

   V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K  S  F  N
AAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA
       1450      1460      1470      1480      1490      1500

   R  G  E  S  *  *
ACCGCGGAGAGTCATAGTAAGAATTC
       1510      1520
```

Fig. 10 cont. (3)



FabD1.3 in pUC19

Fig. 11.

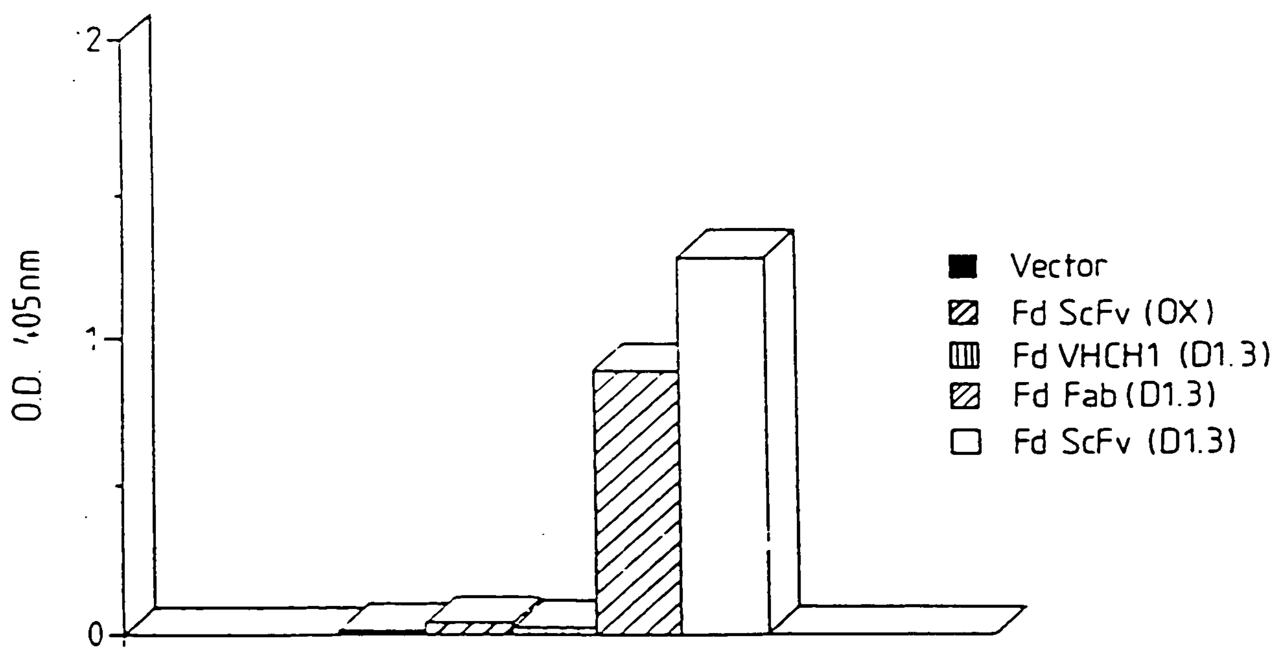


Fig. 12.

Fig.13.

```
Q   V   Q   L   Q   E   S   G   G   G   L   V   Q   P   G   G
CAG GTG CAG CTG CAG GAG TCA GGA GGA GGC TTG GTA CAG CCT GGG GGT
            PstI
S   L   R   L   S   C   A   T   S   G   F   T   F   S   N   Y
TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC AGT AAT TAC

Y   M   G   W   V   R   Q   P   P   G   K   A   L   E   W   L
TAC ATG GGC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT GAG TGG TTG

G   S   V   R   N   K   V   N   G   Y   T   T   E   Y   S   A
GGT TCT GTT AGA AAC AAA GTT AAT GGT TAC ACA ACA GAG TAC AGT GCA

S   V   K   G   R   F   T   I   S   R   D   N   F   Q   S   I
TCT GTG AAG GGG CGG TTC ACC ATC TCC AGA GAT AAT TTC CAA AGC ATC

L   Y   L   Q   I   N   T   L   R   T   E   D   S   A   T   Y
CTC TAT CTT CAA ATA AAC ACC CTG AGA ACT GAG GAC AGT GCC ACT TAT

Y   C   A   R   G   Y   D   Y   G   A   W   F   A   Y   W   G
TAC TGT GCA AGA GGC TAT GAT TAC GGG GCC TGG TTT GCT TAC TGG GGC

Q   G   T    L   V   T   v   s   s   g g g g s g g g g s
CAA GGG ACC  CTG GTC ACC gtc tcc tca ggtggaggcggttcaggcggaggtggctct
                BstEII
g g g g s d   i   E   L   T   Q   T   P   L   S   L   P   V
ggcggtggcggatcggac atc GAG CTC ACC CAA ACT CCA CTC TCC CTG CCT GTC
                          SacI
S   L   G   D   Q   A   S   I   S   C   R   S   S   Q   S   I
AGT CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT

V   H   S   N   G   N   T   Y   L   E   W   Y   L   Q   K   P
GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA CCA
                                                  PstI
G   Q   S   P   K   L   L   I   Y   K   V   S   N   R   F   S
GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT

G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCG GGG ACA GAT TTC ACA

L   K   I   S   R   V   E   A   E   D   L   G   V   Y   Y   C
CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC

F   Q   G   S   H   V   P   Y   T   F   G   G   G   T   K   L
TTT CAA GGT TCA CAT GTT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTC

E   I   K   R
GAG ATC AAA CGG
XhoI
```

Fig. 14.

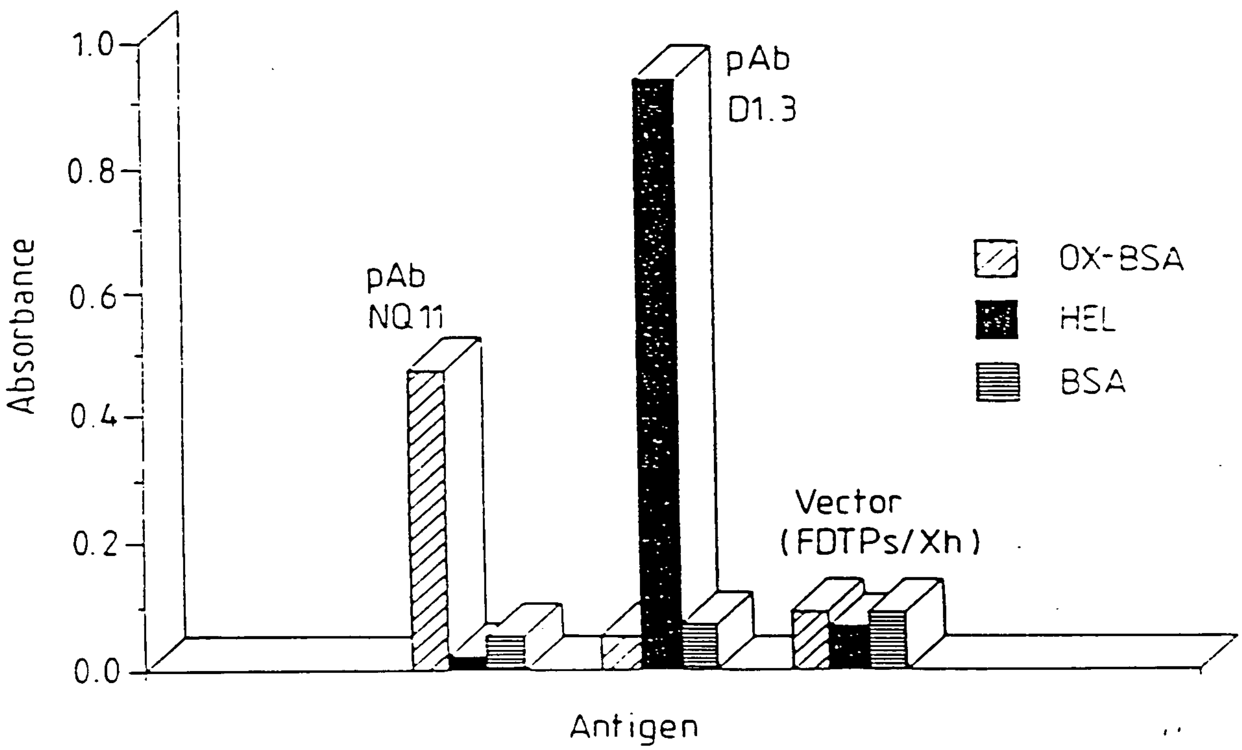


Fig. 15.

5' END

```
                                        R   T   P   E   M   P   V   L
TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG
         ApaL1
```

3' END

```
 K   A   A   L   G   L   K
AAA GCC GCT CTG GGG CTG AAA GCG GCC GCA GAA ACT GTT GAA AGT etc.
                             Not I
```

Fig.16(1)

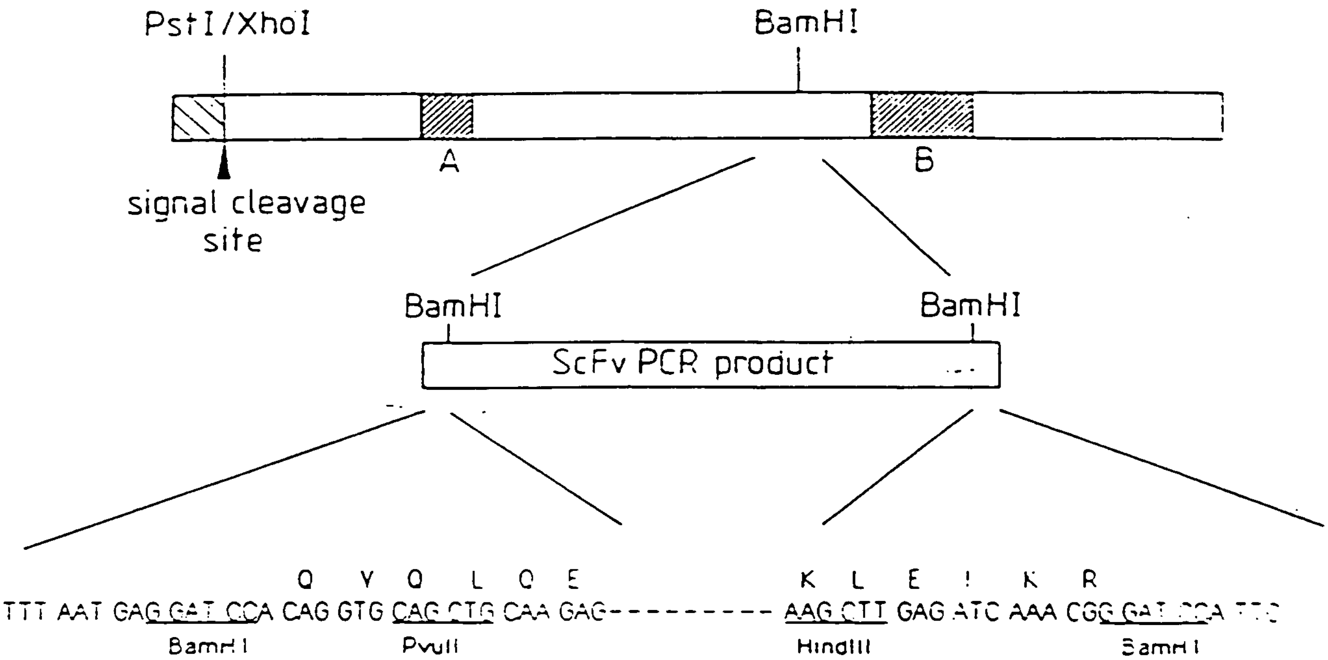


Fig.16(2)

A

(1834) 5' GAG GGT GGT GGC TCT
" " "C " "
" " "C " "
" " "C " ACT 3'(1839)

B

(2284) 5' - GGC GGC GGC TCT
- GGT GGT GGT "
- " GGC GGC "
GAG " " GGC "
" " " GGT "
" " " GGC "
" " " GGT "
- " " GGC " 3'(2379)

Reverse complement of mutagenic oligo G3Bamlink

5' GAG GGT GGC GGA TCC (T above GGA)

GAG GGT GGC GG 3' (T above GG)

Fig.17.

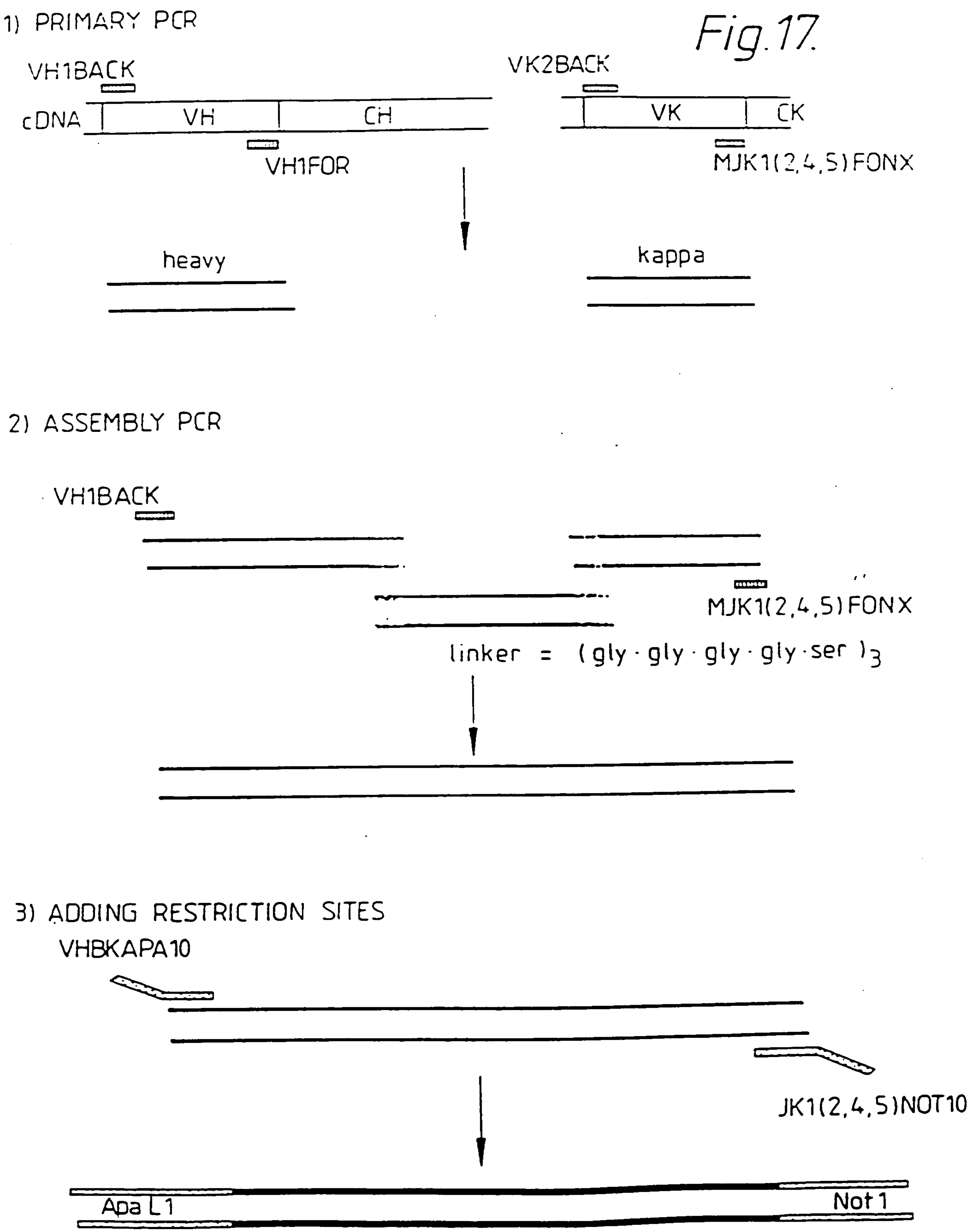

1) PRIMARY PCR
VH1BACK
cDNA
VH
CH
VH1FOR
VK2BACK
VK
CK
MJK1(2,4,5)FONX
heavy
kappa
2) ASSEMBLY PCR
VH1BACK
MJK1(2,4,5)FONX
linker = (gly · gly · gly · gly · ser)3
3) ADDING RESTRICTION SITES
VHBKAPA10
JK1(2,4,5)NOT10
Apa L1
Not 1

Fig.18.

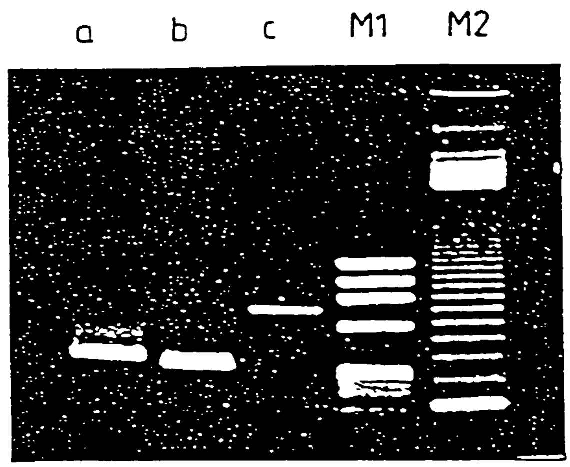


Fig. 19.

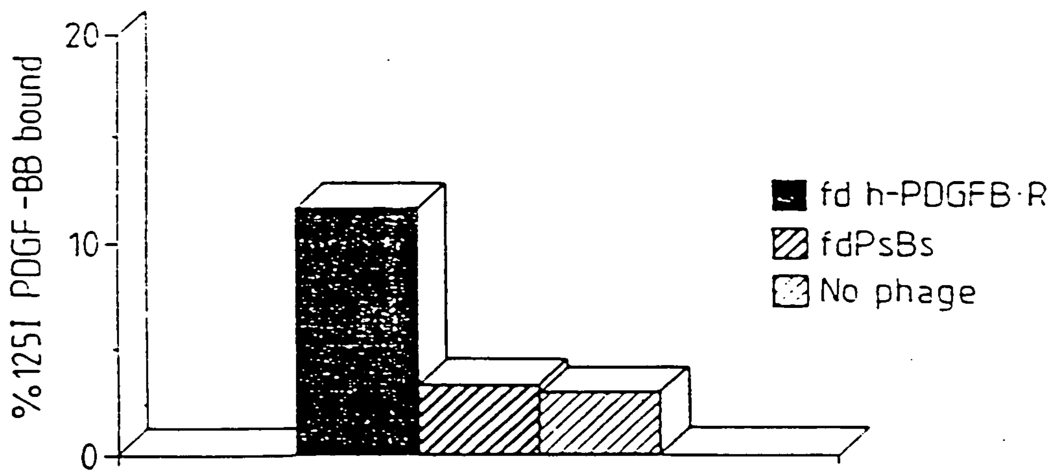

20
10
0
%125I PDGF-BB bound
fd h-PDGFB-R
fdPsBs
No phage

Fig. 20.

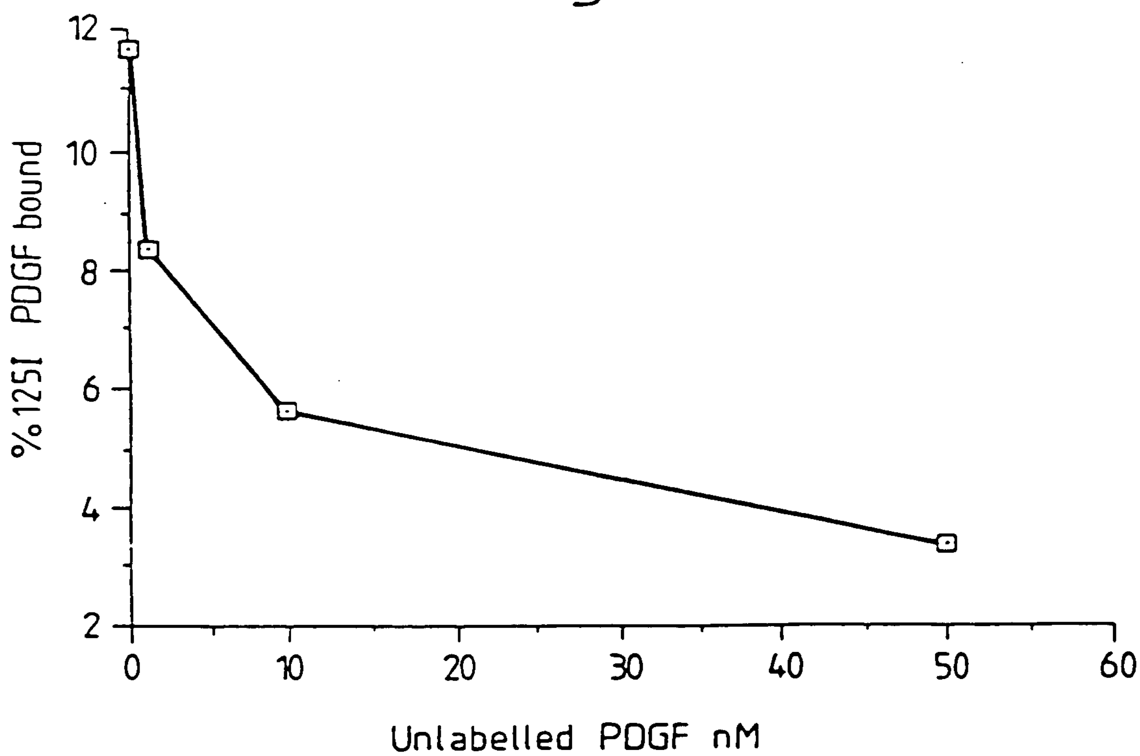

12
10
8
6
4
2
%125I PDGF bound
0
10
20
30
40
50
60
Unlabelled PDGF nM

Fig. 21.

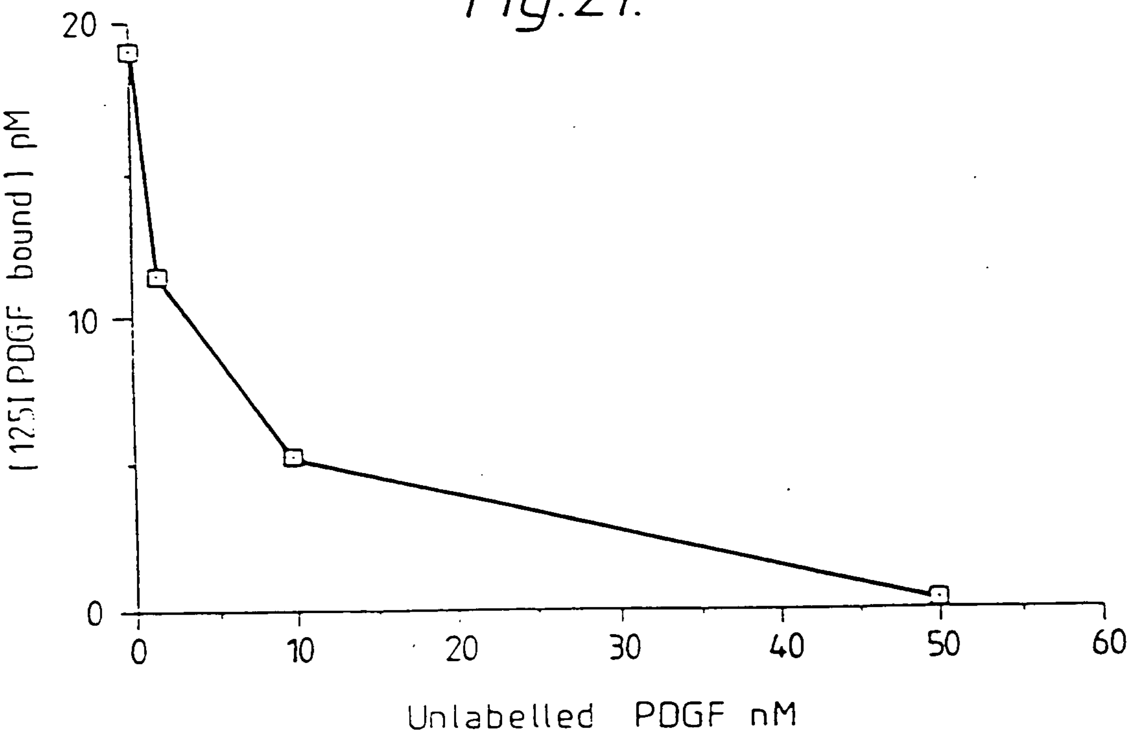

20
10
0
[125I PDGF bound] pM
0
10
20
30
40
50
60
Unlabelled PDGF nM

Fig. 22.

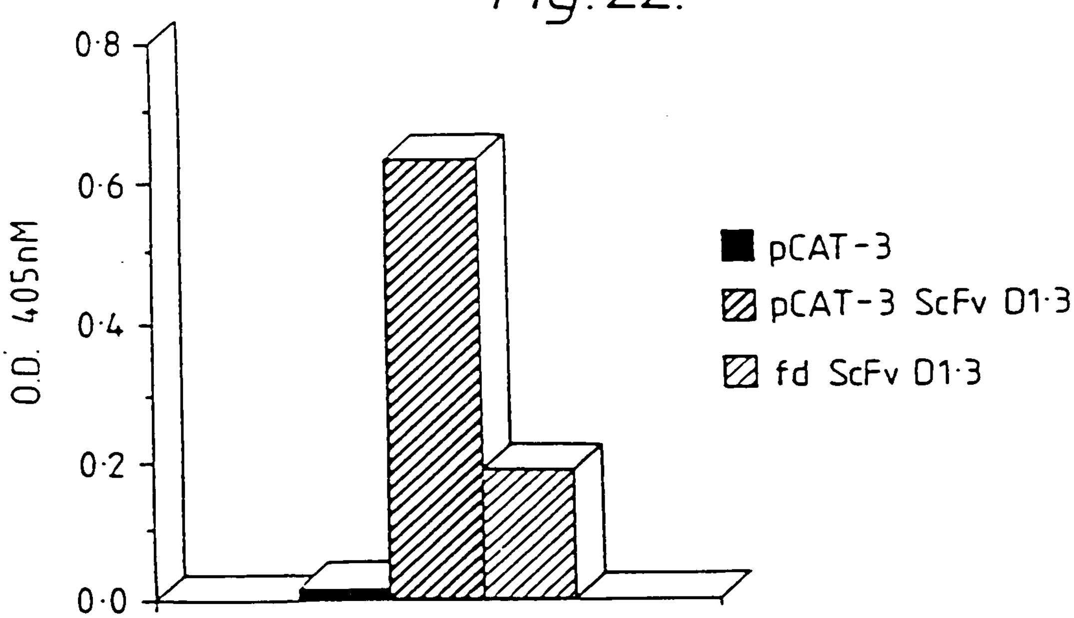

0·8
0·6
0·4
0·2
0·0
O.D. 405nM
pCAT-3
pCAT-3 ScFv D1·3
fd ScFv D1·3

Fig. 23.

d

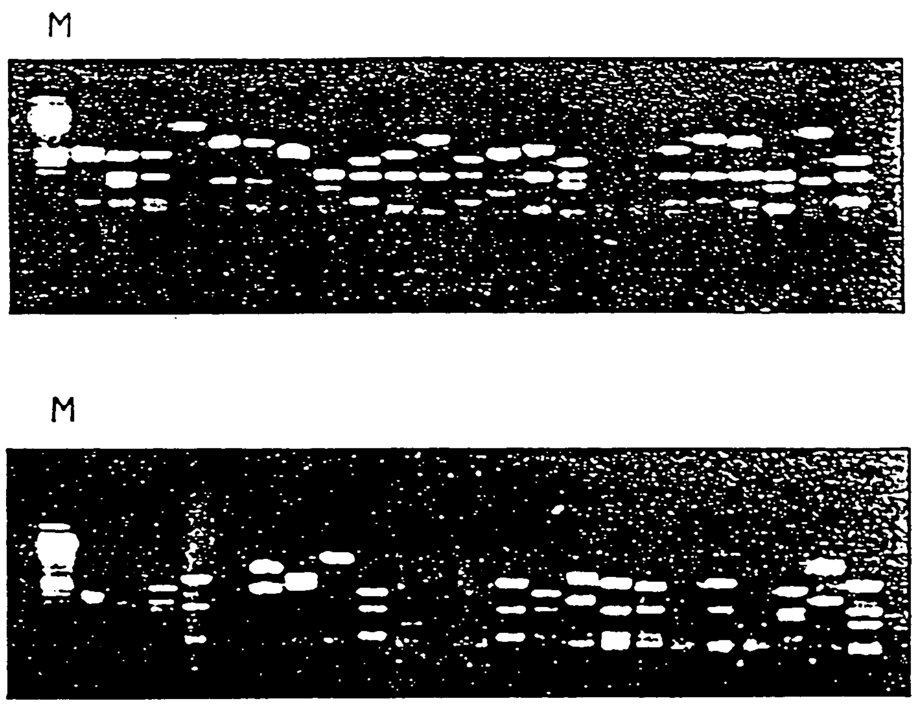


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Fig. 24.

VH sequences

from combinatorial library:

		CDR1		CDR2		CDR3		
A	QVQLQQSGAELARPGASVKMSCKASGYTFT	SYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAN	RYGAY	WGQGTTVTVSS x4	
B	QVKLQQSGAELAKPGASVKMSCKASGYTFT	RDWMH	WLKQRPGQGLEWIG	YINPSTGYTSYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGLY	WGQGTTVTVSS x9	
C	QVQLQQSGPELVKPGASVKMSCKASGYTFT	SYVMH	WVKQKPGQGLEWIG	YINPYNDGTKYNEKFKG	KATLTSDKSSSTAYMELSSLTSEDSAVYYCAI	YRGFPY	WGQGTTVTVSS x3	
D	QVQLQQSGPELVKPGASVKISCKASGYSFT	GYFMH	WVKQSHGKSLEWIG	RINPYNGDTFYNQKFKG	KATLTVDKSSSTAHMELLSLTSEDSAVYYCVG	ITTRFAY	WGQGTTVTVSS x1	
E	QVQLQESGPGLVAPSQSLSITCTVSGFSLT	SYGVH	WVRQPPGKGLEWLG	VIWAGGSTNYNSALMS	RLSISKDNSKSQVFLKMNSLQTDDTAMYYCAR	DRGDY	WGQGTTVTVSS	VHox1
F	QVQLQQSGPELAKPGASVKMSCKASGYTFT	SYLMH	WVKQRPGQGLKWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS	
G	QVKLQQSGAELVRPGASVKLSCKASGYSFS	RYLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	SATLTADKSSNTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS	
H	QVQLQQSGPELMKPGASVKISCKASGYSFS	NWYMH	WVKQSHGKSLEWIG	YIAPFNGGTTYNQKFKG	KATLTVDRSSSTAYMHLSSLTSEDSAVYYCAT	DYGRD	WGQGTTVTVSS	

from hierarchical library VH-rep x Vκ-d:

I	QVKLQQSGPELARPGVSVKMSCKASGYTFT	SYAMH	WVKQSQSKSLEWIG	VISTYNGNTNYNQKFKG	KATMTVDKSSSTAYMELARLTSEDSAIYYCAR	DYGDY	WGQGTTVTVSS
J	QVKLQQSGAELARPGASVKMSCKASGYTFT	RYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DRGAY	WGQGTTVTVSS
K	QVKLQQSGAELAKPGASVKMSCKASGYTFT	RDWMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGLY	WGQGTTVTVSS x1
L	QVQLQQSGLELAKPGASVKMSCKASGYTFT	NYLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS x2
M	QVKLQQSGAELAKPGASVKMSCKASGYTFT	NYWMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYF	WGQGTTVTVSS
N	QVKLQQSGAELVKPGASVKLSCKTSGYTFT	SYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSDDSAVYYCAR	DYGYY	WGQGTTVTVSS
O	QVQLQQSGAELVKPGASVKMSCSASGYTFT	GHLMH	WVKQRPGQGLEWIG	YINPRTGYTSYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGAY	WGQGTTVTVSS
P	QVQLQQSGAELAKPGASVKMSCKASGYTFT	SYWMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS
Q	QVKLQQSGAELAKPGASVKMSCKATGYTFT	SYLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATFTADKSSSTAYMQLSGLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS
R	QVKLQQSGAELAKPGASVKMSCKASGYTFT	SYVMH	WLKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGLY	WGQGTTVTVSS x2
S	QVQLQQSGAELARPGASVKMSCKASGYTFT	TPLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS x4
T	QVQLQQSGAELAKPGASVKMSCKASGYTFT	SYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS
U	QVKLQQSGAELARPGASVKMSCKASGYTFT	SYTMH	WLKQRPGQGLEWIG	YINPTTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS
V	QVKLQQSGAELAKPGASVKMSCKASGYTFT	RDWMH	WLKQRPGQGLEWIG	YINPSTGYTSYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGLY	WGQGTTVTVSS

Fig.24 cont.

Vκ sequences

from combinatorial library:

		CDR1		CDR2		CDR3			
a	DIELTQSPSSLSASLGERVSLTC	RASQEISGYLS	WLQQKPDGSIKRLIY	AASTLDS	GVPKRFSGSRSGSDYSLTISSLESEDFADYYC	LQYASYPT	FGAGTKLEIKRA x1	V	ox-like
b	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WYQQKSGASPKVWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGAGTKLEIKRA x1	IV	ox-like
c	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPLT	FGAGTKLEIKRA x2	IV	ox-like
d	DIELTQSPTIMAASPGEKITITC	SASSSISSNYLH	WFQQKPGFSPKLLIS	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSTIPPT	FGSGTKLEIKRA x9	IV	ox-like
e	DIELTQSPAIMSASPGEKVTITC	SASSSVNYMH	WFQQKPGTSPKLWIY	STSNLAS	GVPTRFSGSGSGTSYSLTISRMEAEDAATYYC	QQRSSYPPT	FGSGTKLEIKRA x4	VI	ox-like?
f	DIELTQSPAIMSAFPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQFSGNPLT	FGAGTKLELKRA	VI	Vκox1
g	DIELTQSPAIMSASPGEKVTMTC	SASSSINYMH	WYQQKPGASPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	HQRHAYPWT	FGGGTKLEIKRA	VI	ox-like?

from hierarchical library VH-B x Vκ-rep:

h	DIELTQSPAIMSASPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSNPLT	FGAGTKLELKRA x4	IV/VI	Vκox1
i	DIELTQSPAIMSASPGEKVTITC	SASSSVSYIH	WFQQKPGTSPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISRMEAEDAATYYC	QQYHSYPLT	FGAGTKLEIKRA	V	ox-like?
j	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WFQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPLT	FGGGTKLEIKRA	V	ox-like
k	DIELTQSPTTMAASPGDHITITC	SATSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPPRFSGSGSGTSYSLTIGAMEAEDVATYYC	QQGSSIPYT	FGAGTKLEIKRA	V	ox-like
l	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPYT	FGGGTKLEIKRA	V	ox-like
m	DIELTQSPTIMAASPGEKITITC	SASSSISSNNLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSGIPYT	FGGGTKLEIKRA	V	ox-like
n	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPFT	FGGGTKLEIKRA	V	ox-like
o	DIELTQSPAIMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSFKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPYT	FGGGTKLEIKRA x2	V	ox-like
p	DIELTQSPAIMSASPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDVATYYC	QQWSSNPLT	FGAGTKLEIKRA x2	IV/VI	Vκox1
q	DIELTQSPAIMSASPGDKVTLTC	SASSSVRYVN	WFQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWTSNPPT	FGGGTKLEIKRA	IV/VI	Vκox1
r	DIELTQSPAIMSASPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSNPLT	FGAGTKLEIKRA	IV/VI	Vκox1
s	DIELTQSPAIMSASPGEKVTMTC	RASSSVTSSYLH	WYQQKSGASPKLWVY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGAGTKLELKRA	IV/VI	ox-like
t	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISRMEAEDAATYYC	QQRSSYPLT	FGAGTKLEIKRA	IV/VI	ox-like
u	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGAGTKLELKRA	IV/VI	ox-like
v	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WFQQKSGASPKLWIY	STSNLPS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGGGTKLEIKRA	IV/VI	ox-like
c	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPLT	FGAGTKLEIKRA x1		

Fig. 25.

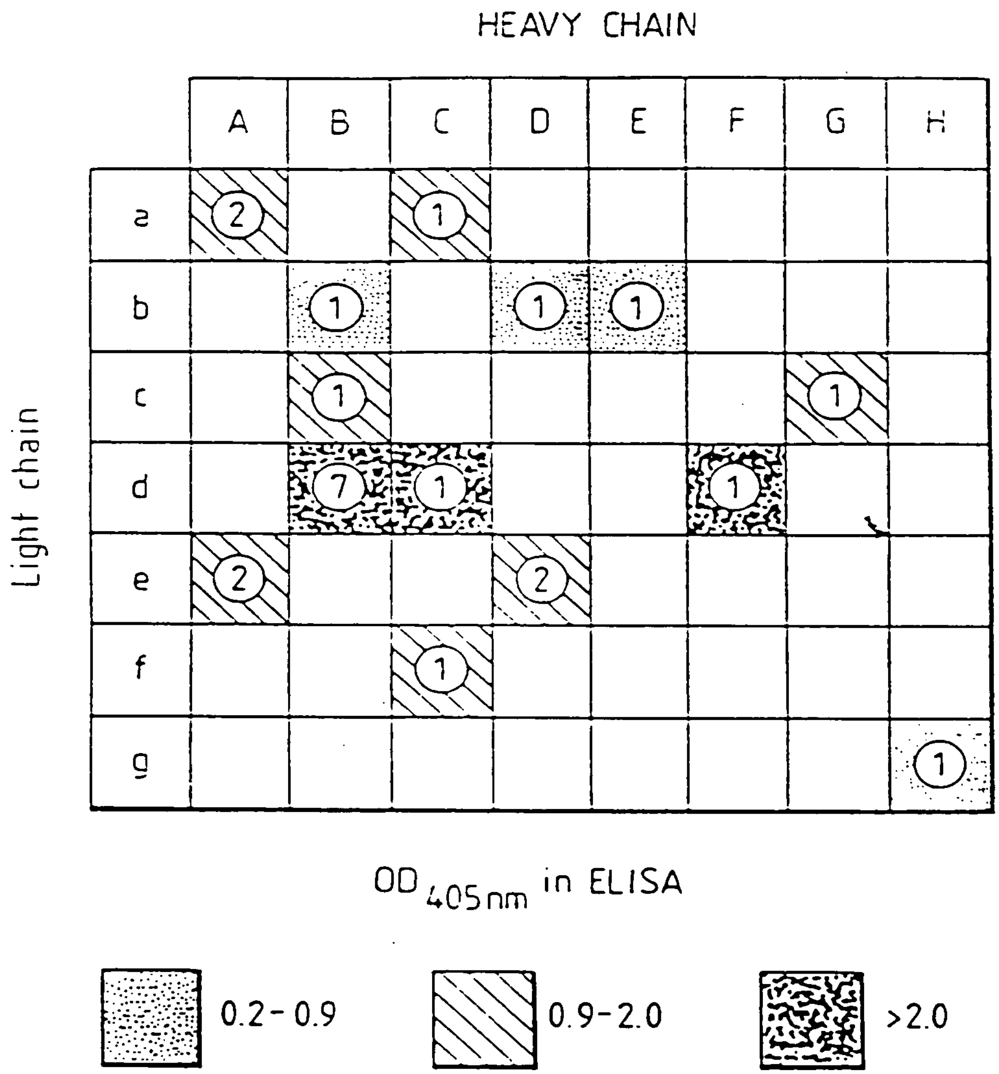
HEAVY CHAIN
A
B
C
D
E
F
G
H
Light chain
a
b
c
d
e
f
g
2
1
1
1
1
1
1
7
1
1
2
2
1
1
OD 405nm in ELISA
0.2-0.9
0.9-2.0
>2.0

Fig.26(a)

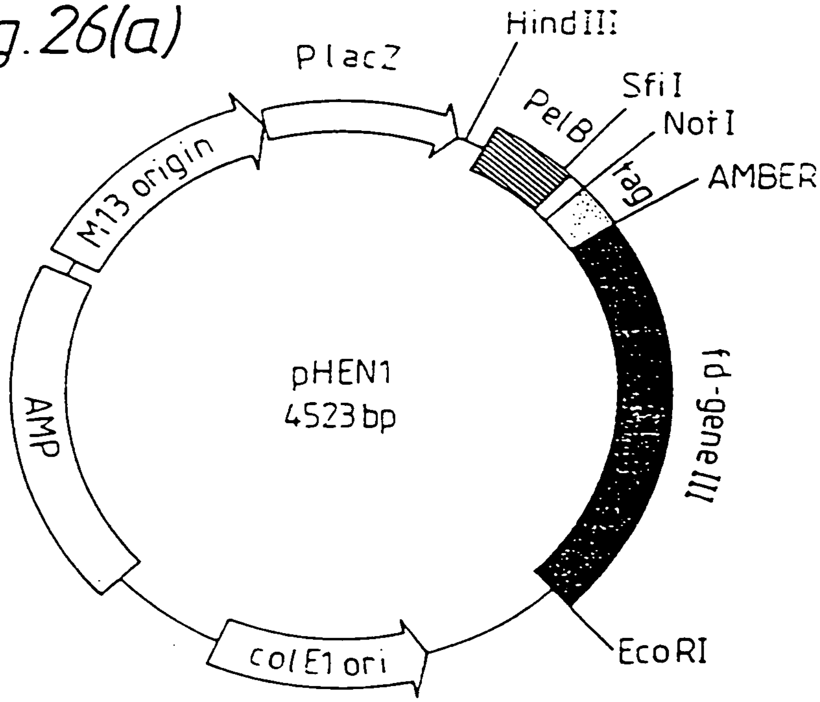

Fig.26(b)

```
···———— PelB leader ————┼- - - - - - - - -pHEN1 polylinker- - - - -
                       -1  +1
 L  L  A  A  Q  P  A  M  A  Q  V  Q  L  Q  V  D  L  E  I  K  R
--TTACTCGCGGCCCAGccggccatggcccaggtgcagctgcaggtcgacctcgagatcaaacgg
        SfiI        NcoI          PstI      SalI    XhoI

------├ ————— c-myc tag ——————————┤      ├---fd-gene III----
 A  A  A  E  Q  K  L  I  S  E  E  D  L  N  G  A  A (E) T  V  E
gcggccGCAGAACAAAAACTCATCTCAGAAGAGGATCTGAATGGGGCCGCATAGACTGTTGAA--
                                                 amber
NotI
```

Fig. 27.

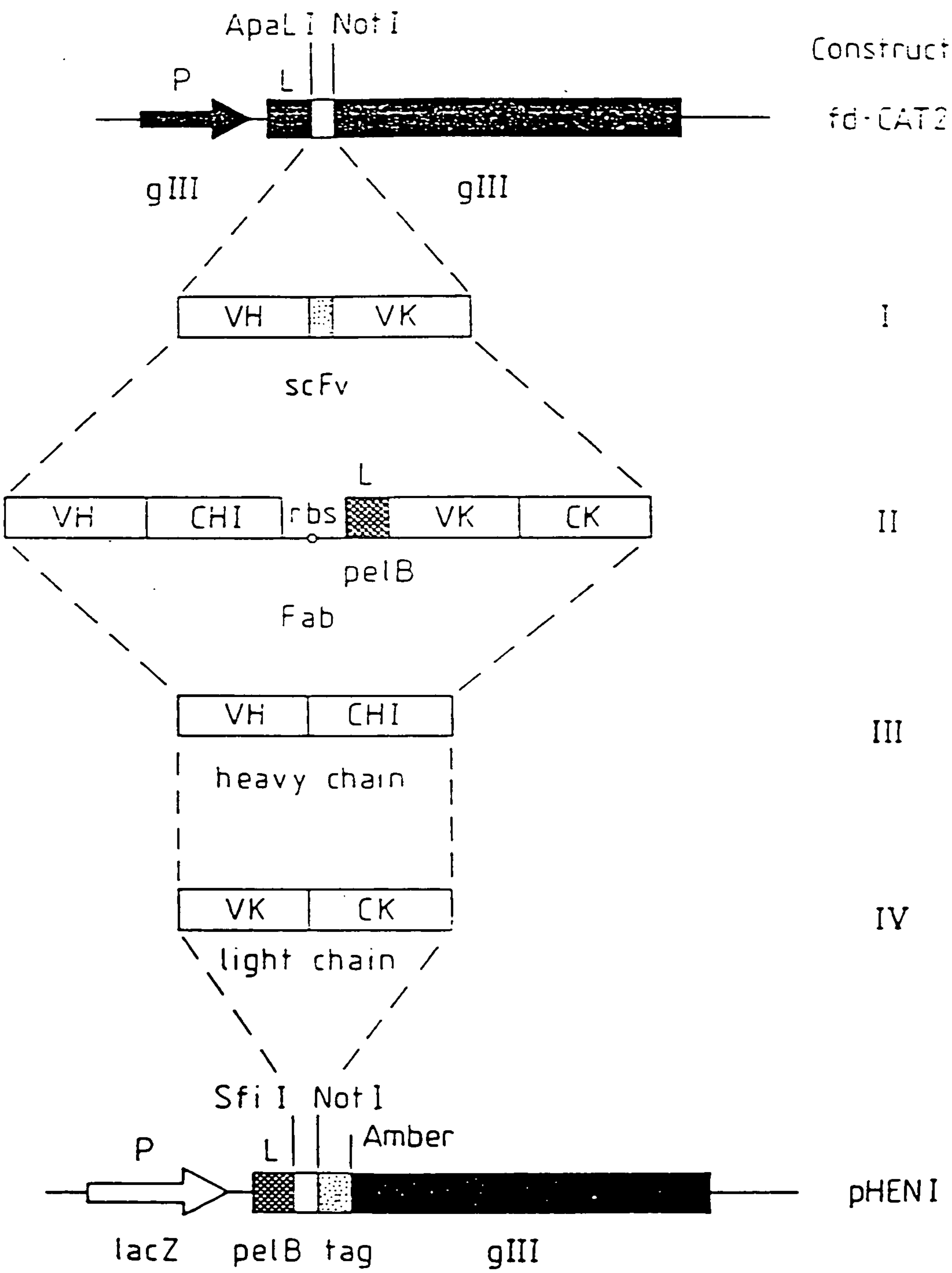
ApaL I
Not I
Construct
P
L
fd-CAT2
gIII
gIII
VH
VK
I
scFv
L
VH
CHI
rbs
VK
CK
II
pelB
Fab
VH
CHI
III
heavy chain
VK
CK
IV
light chain
Sfi I
Not I
Amber
P
L
pHEN I
lacZ
pelB
tag
gIII

Fig. 28.

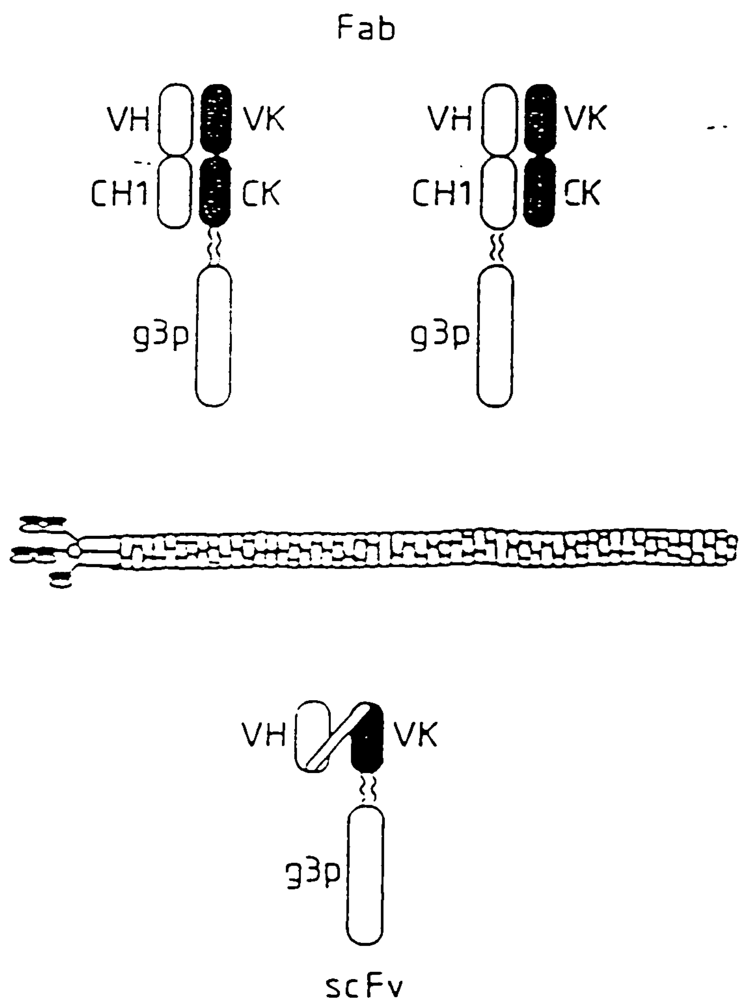

Fig.29.

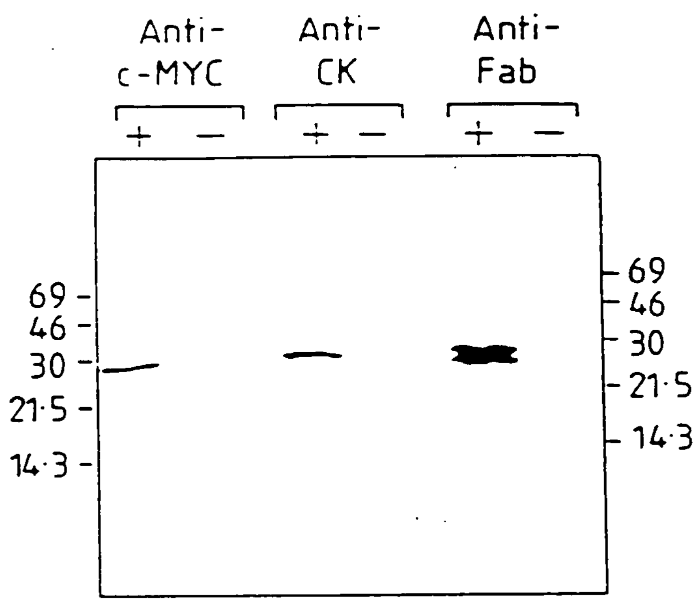

Fig.30.

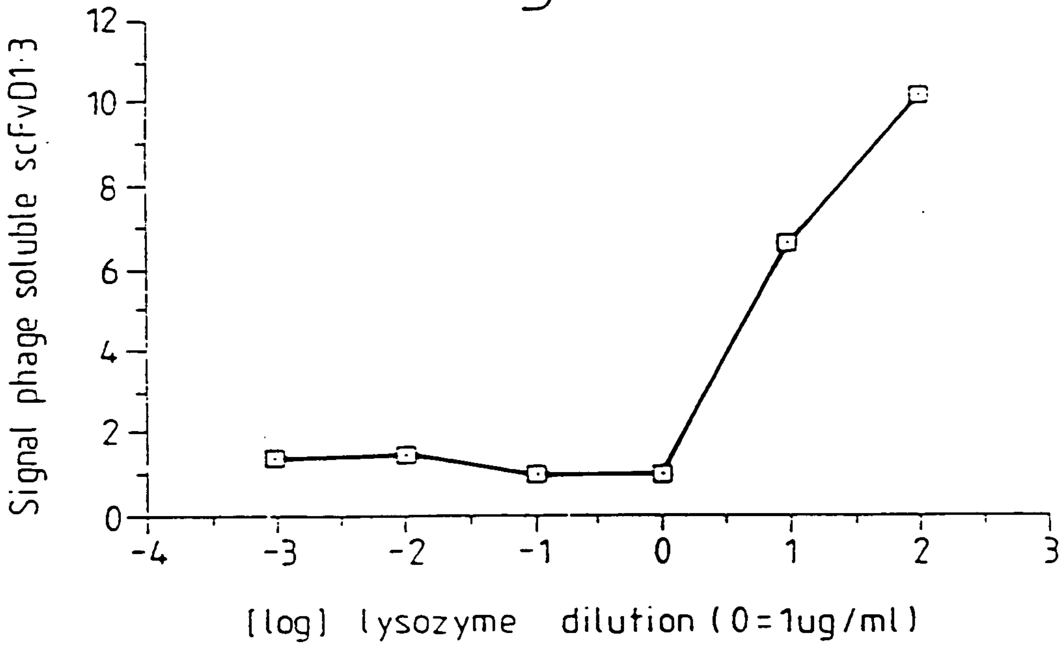
Signal phage soluble scFvD1·3
12
10
8
6
4
2
0
-4
-3
-2
-1
0
1
2
3
[log] lysozyme dilution (0=1ug/ml)

Fig.31.

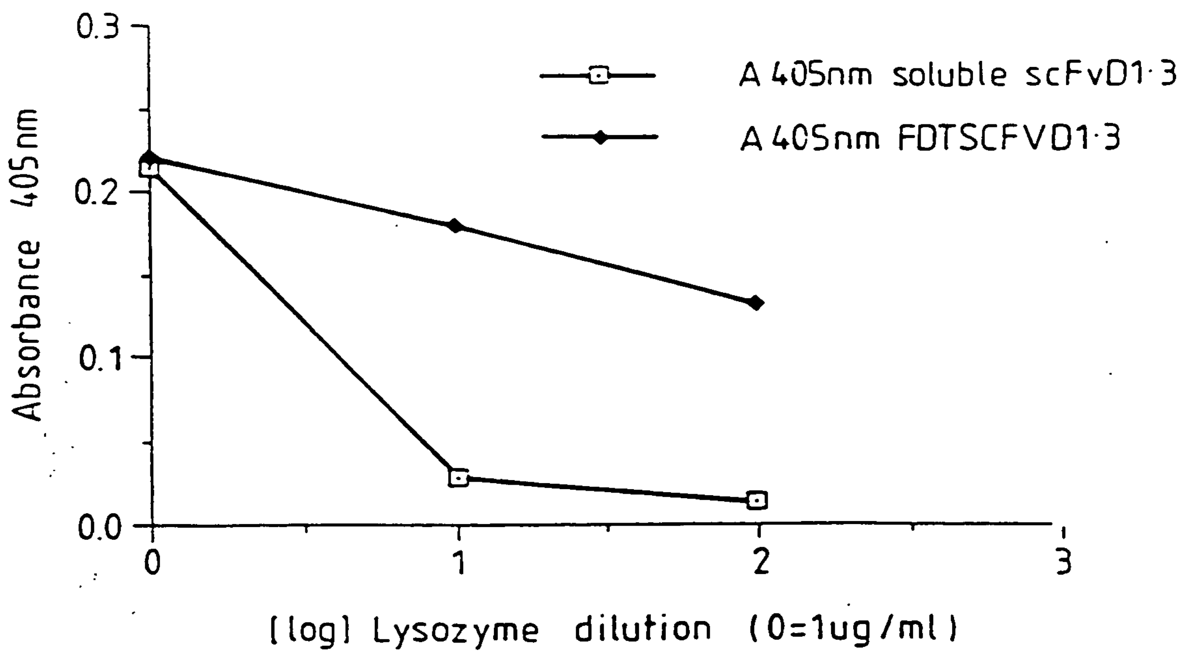
Absorbance 405nm
0.3
0.2
0.1
0.0
0
1
2
3
A 405nm soluble scFvD1·3
A 405nm FDTSCFVD1·3
[log] Lysozyme dilution (0=1ug/ml)

Fig. 32.

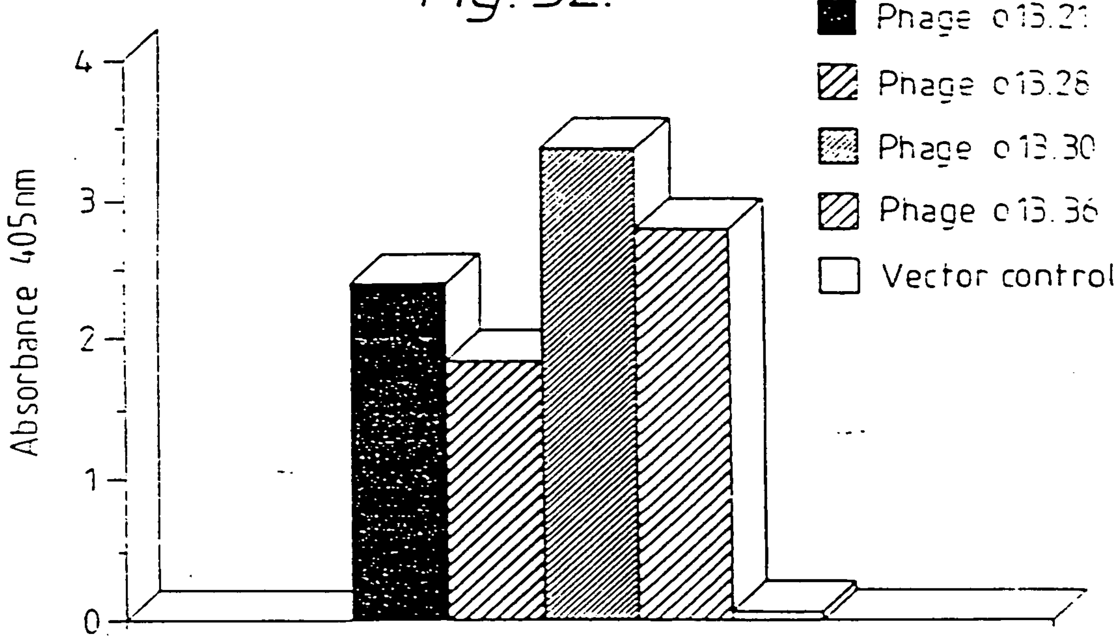
Absorbance 405nm
4
3
2
1
0
Phage o13.21
Phage o13.28
Phage o13.30
Phage o13.36
Vector control

Fig. 33.

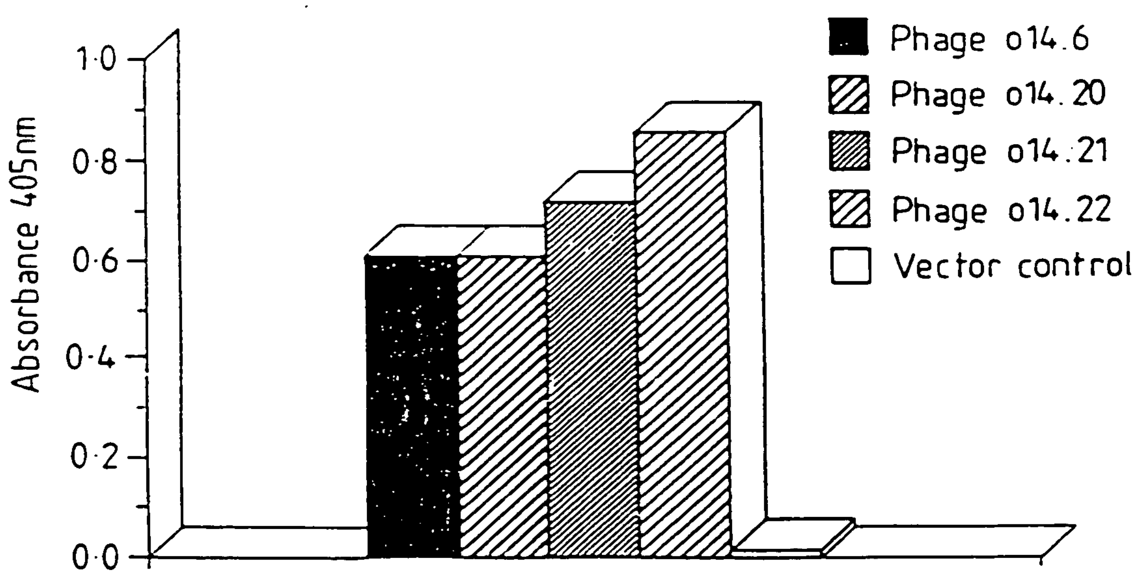
Absorbance 405nm
1·0
0·8
0·6
0·4
0·2
0·0
Phage o14.6
Phage o14.20
Phage o14.21
Phage o14.22
Vector control

Fig. 34.

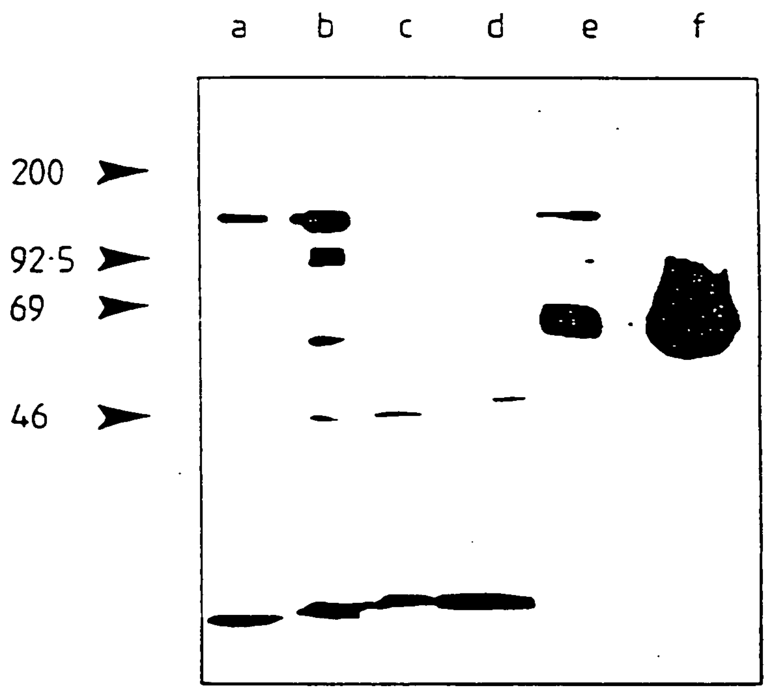

Fig. 35.

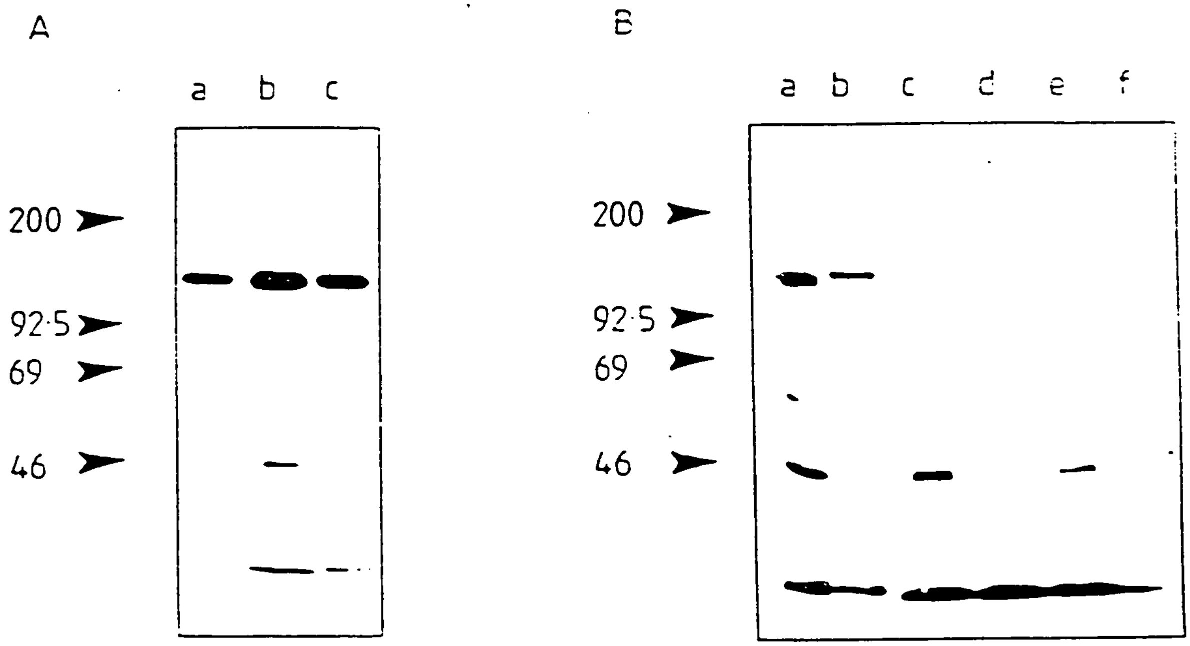
A
a b c
200
92·5
69
46
B
a b c d e f
200
92·5
69
46

Fig. 36.

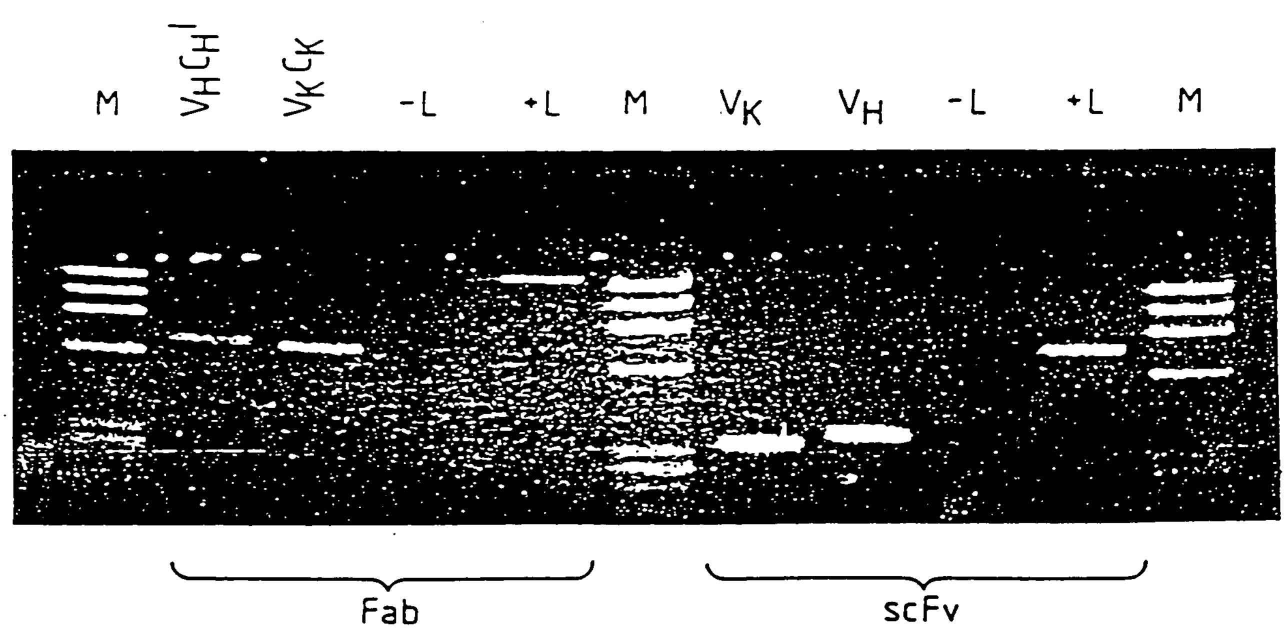
M
V_HC_H1
V_KC_K
-L
+L
M
V_K
V_H
-L
+L
M
Fab
scFv

Fig.37.

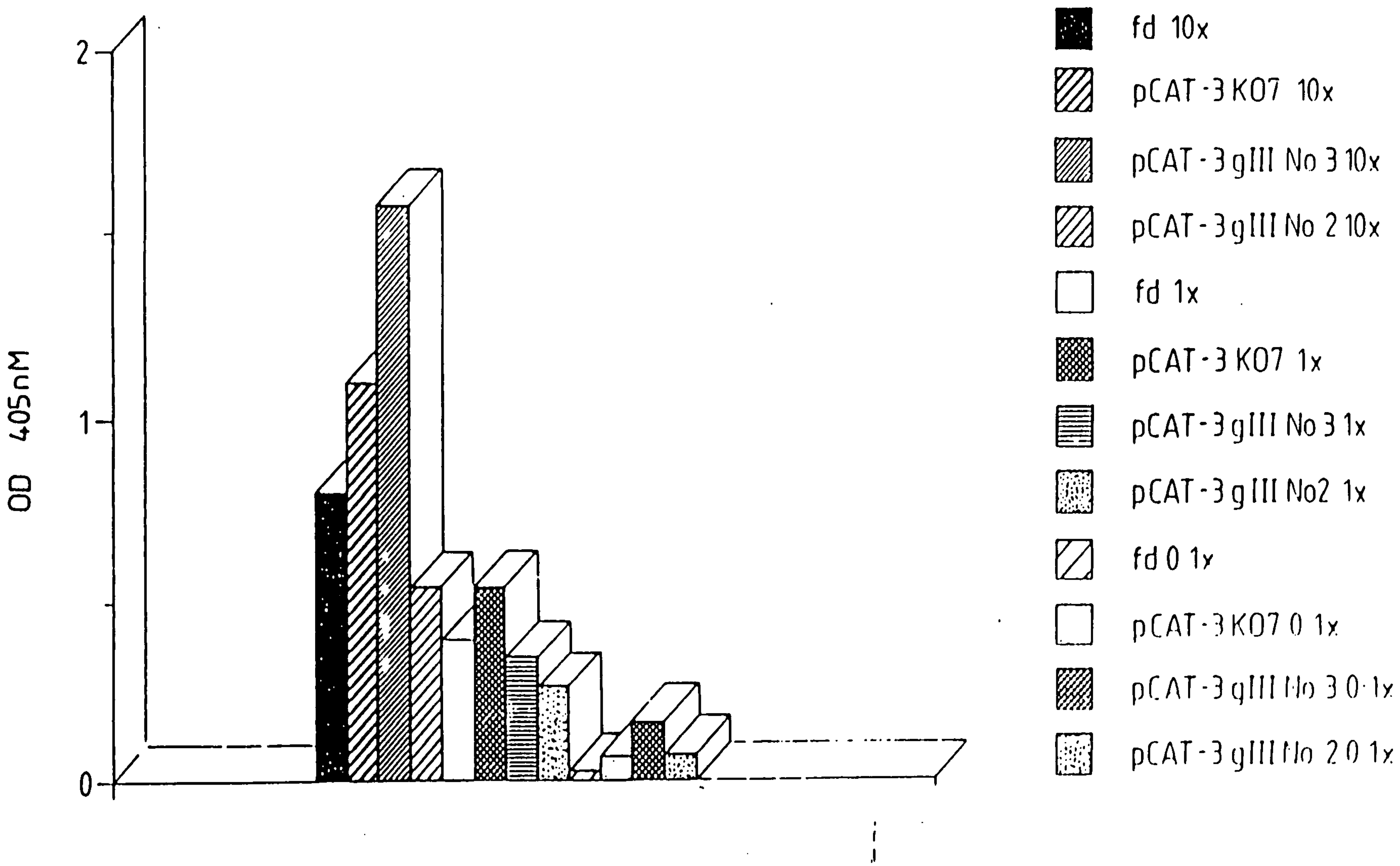
fd 10x
pCAT-3 KO7 10x
pCAT-3 gIII No 3 10x
pCAT-3 gIII No 2 10x
fd 1x
pCAT-3 KO7 1x
pCAT-3 gIII No 3 1x
pCAT-3 gIII No2 1x
fd 0 1x
pCAT-3 KO7 0 1x
pCAT-3 gIII No 3 0·1x
pCAT-3 gIII No 2 0 1x
2
1
0
OD 405nM

Fig. 38.

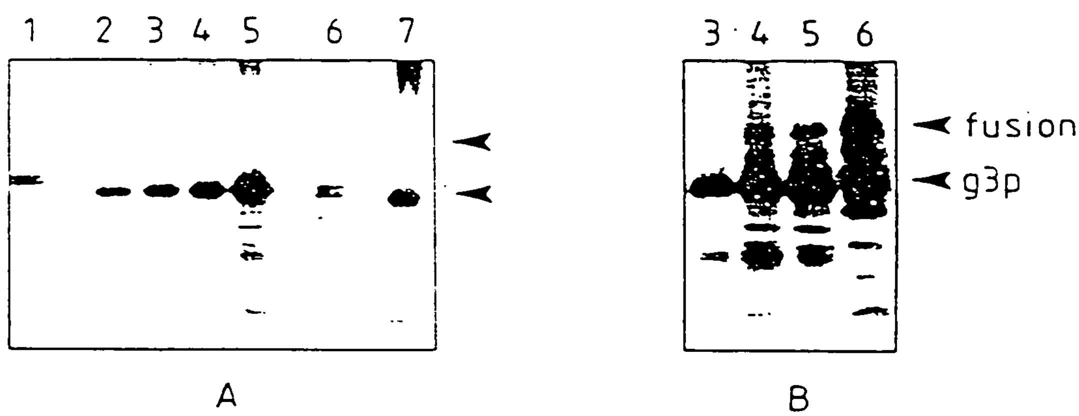

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Fig. 39.

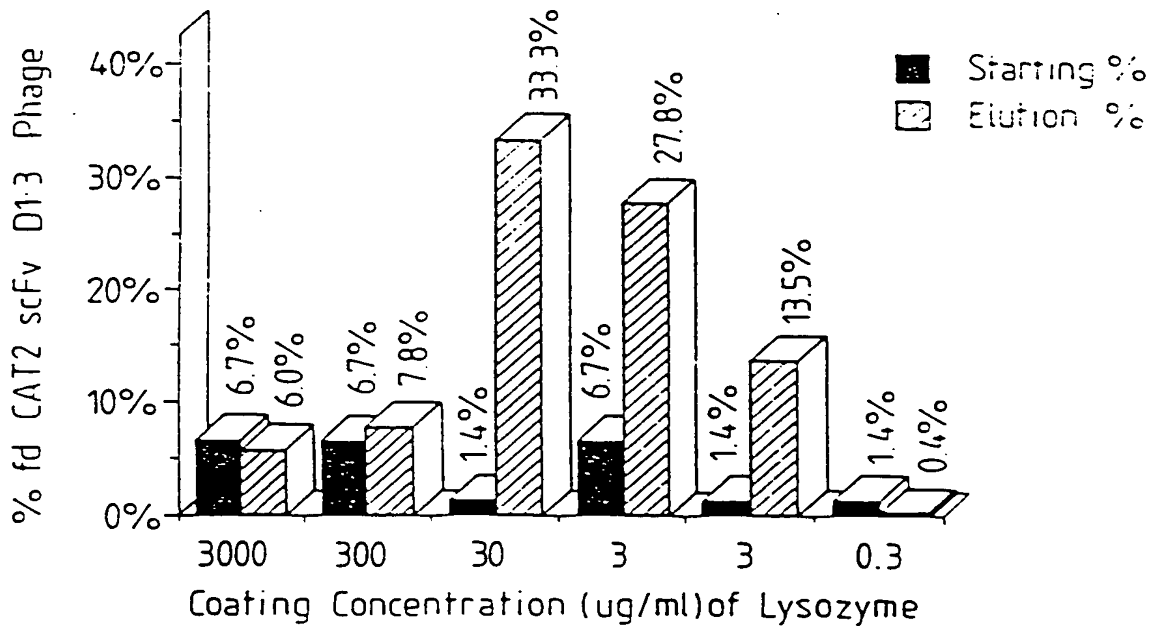

Fig. 40.

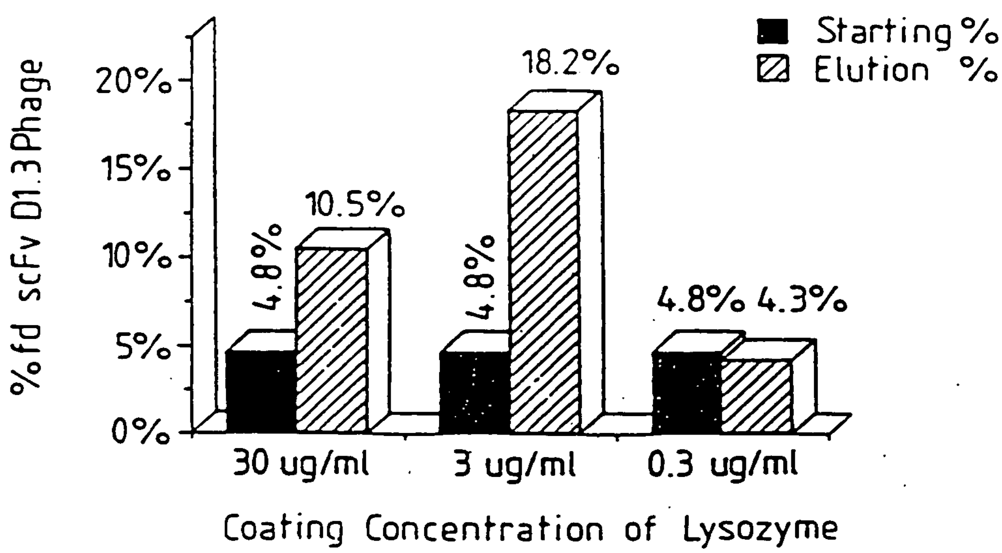

Fig. 41.

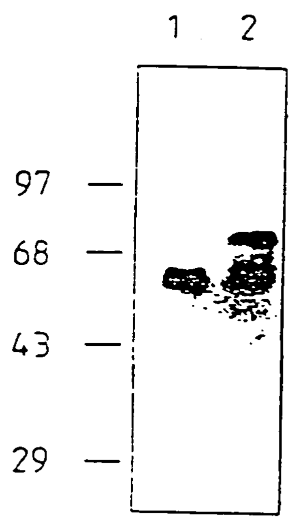

Fig. 42.

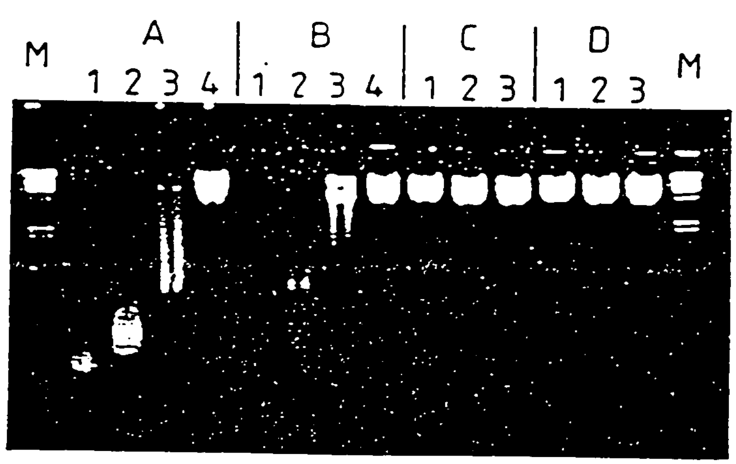

Fig. 43.

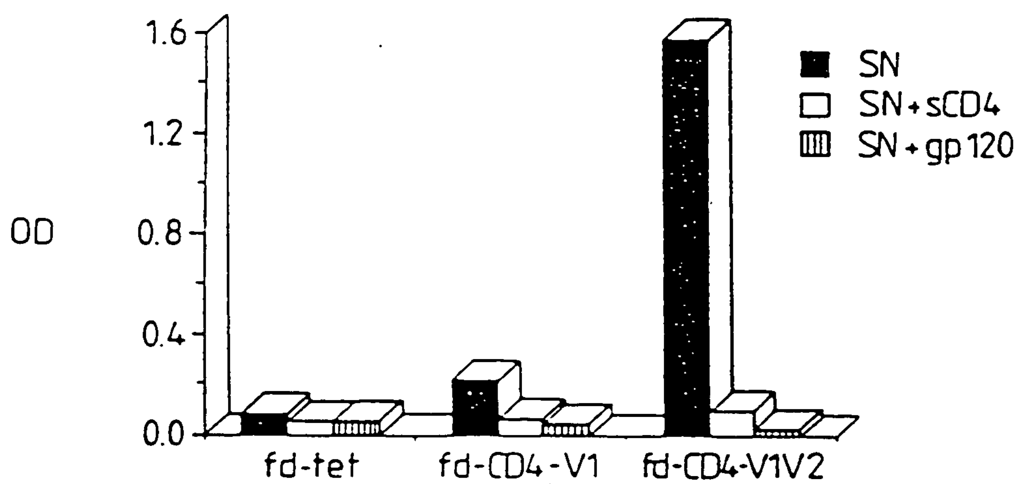

Fig. 44 (i)

```
10        20        30        40        50        60        70        80        90
TTCTATTCTCACNGTGCNCAGGTCCAGCTGCAGCAGTCTGGGGCTGAGCTTGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCT
AAGATAAGAGTGTCACGTGTCCAGGTCGACGTCGTCAGACCCCGACTCGAACACTTCGGACCCCGAAGTCACTTCGACAGGACGTTCCGA
PheTyrSerHisSerAlaGlnValGlnLeuGlnGlnSerGlyAlaGluLeuValLysProGlyAlaSerValLysLeuSerCysLysAla

100       110       120       130       140       150       160       170       180
TCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACGAGGCCTTGAGTGGATTGGAAGGATTGATCCTAAT
AGACCGATGTGGAAGTGGTCGATGACCTACGTGACCCACTTCGTCTCCGGACCTGCTCCGGAACTCACCTAACCTTCCTAACTAGGATTA
SerGlyTyrThrPheThrSerTyrTrpMetHisTrpValLysGlnArgProGlyArgGlyLeuGluTrpIleGlyArgIleAspProAsn

190       200       210       220       230       240       250       260       270
AGTGGTGGTACTAAGTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAACCCTCCAGCACAGCCTACATGCAGCTCAGC
TCACCACCATGATTCATGTTACTCTTCAAGTTCTCGTTCCGGTGTGACTGACATCTGTTTGGGAGGTCGTGTCGGATGTACGTCGAGTCG
SerGlyGlyThrLysTyrAsnGluLysPheLysSerLysAlaThrLeuThrValAspLysProSerSerThrAlaTyrMetGlnLeuSer

280       290       300       310       320       330       340       350       360
AGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGCAAGATACGACTACGGTAGTAGCTACTACTTTGACTACTGGGGCCAAGGGACC
TCGGACTGTAGACTCCTGAGACGCCAGATAATAACACGTTCTATGCTGATGCCATCATCGATGATGAAACTGATGACCCCGGTTCCCTGG
SerLeuThrSerGluAspSerAlaValTyrTyrCysAlaArgTyrAspTyrGlySerSerTyrTyrPheAspTyrTrpGlyGlnGlyThr

370       380       390       400       410       420       430       440       450
ACGGTCACCGTCTCCTCNGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCCCAGGCTGTTGGGACACAGGAATCTGCA
TGCCAGTGGCAGAGGAGTNCCACCTCCGCCAAGTCCGCCTCCACCGAGACCGCCACCGCCTAGGGTCCGACAACCCTGTGTCCTTAGACGT
ThrValThrValSerSerGlyGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySerGlnAlaValGlyThrGlnGluSerAla

460       470       480       490       500       510       520       530       540
CTCACCACATCACCTGGTGAAACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACAACTAGTAACTATGCCAACTGGGTCCAA
GAGTGGTGTAGTGGACCACTTTGTCAGTGTGAGTGAACAGCGAGTTCATGACCCCGACAATGTTGATCATTGATACGGTTGACCCAGGTT
LeuThrThrSerProGlyGluThrValThrLeuThrCysArgSerSerThrGlyAlaValThrThrSerAsnTyrAlaAsnTrpValGln

550       560       570       580       590       600       610       620       630
GAAAAACCAGATCATTTATTCACTGGTCTAATAGGTGGTACCAACAACCGAGCTCCAGGTGTTCCTGCCAGATTCTCAGGCTCCCTGATT
CTTTTTGGTCTAGTAAATAAGTGACCAGATTATCCACCATGGTTGTTGGCTCGAGGTCCACAAGGACGGTCTAAGAGTCCGAGGGACTAA
GluLysProAspHisLeuPheThrGlyLeuIleGlyGlyThrAsnAsnArgAlaProGlyValProAlaArgPheSerGlySerLeuIle
```

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Fig. 44 (ii)

```
        640       650       660       670       680       690       700 C  G 710       720
GGAGACAAGGCTGCCCTCACCATCACAGGGGCACAGACTGAGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTG
CCTCTGTTCCGACGGGAGTGGTAGTGTCCCCGTGTCTGACTCCTACTCCGTTATATAAAGACACGAGATACCATGTCGTTGGTAACCCAC
GlyAspLysAlaAlaLeuThrIleThrGlyAlaGlnThrGluAspGluAlaIleTyrPheCysAlaLeuTrpTyrSerAsnHisTrpVal

     730       740       750       760       770
TTCGGTGGAGGAACCAAACTGACTGTCCTCGAGATCAAACGGGCGGCCGC
AAGCCACCTCCTTGGTTTGACTGACAGGAGCTCTAGTTTGCCCGCCGGCG
PheGlyGlyGlyThrLysLeuThrValLeuGluIleLysArgAlaAla
```

Fig. 45.

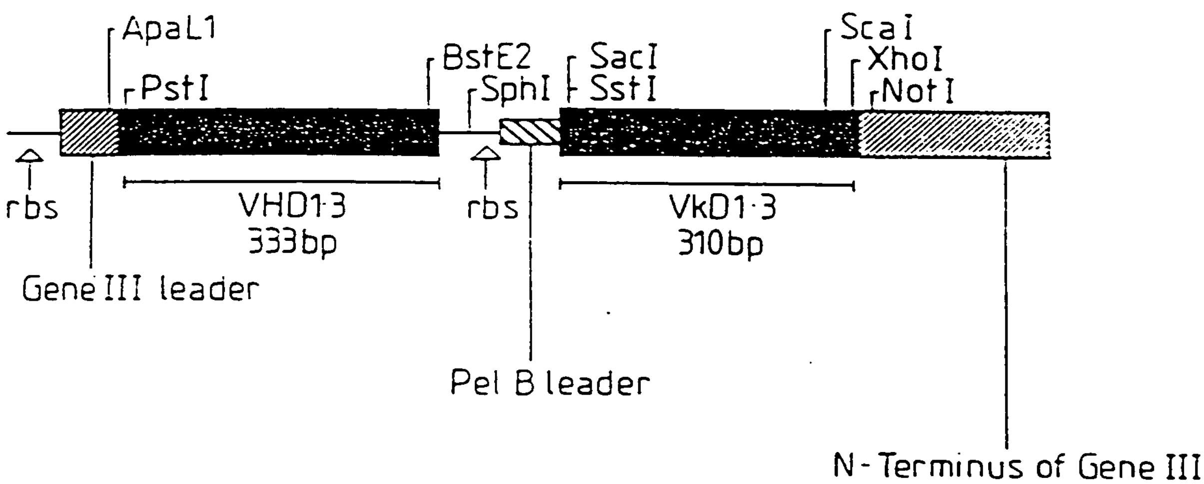

ApaL1
PstI
BstE2
SphI
SacI
SstI
ScaI
XhoI
NotI
rbs
VHD1·3
333bp
rbs
VkD1·3
310bp
Gene III leader
Pel B leader
N-Terminus of Gene III

Fig. 46.

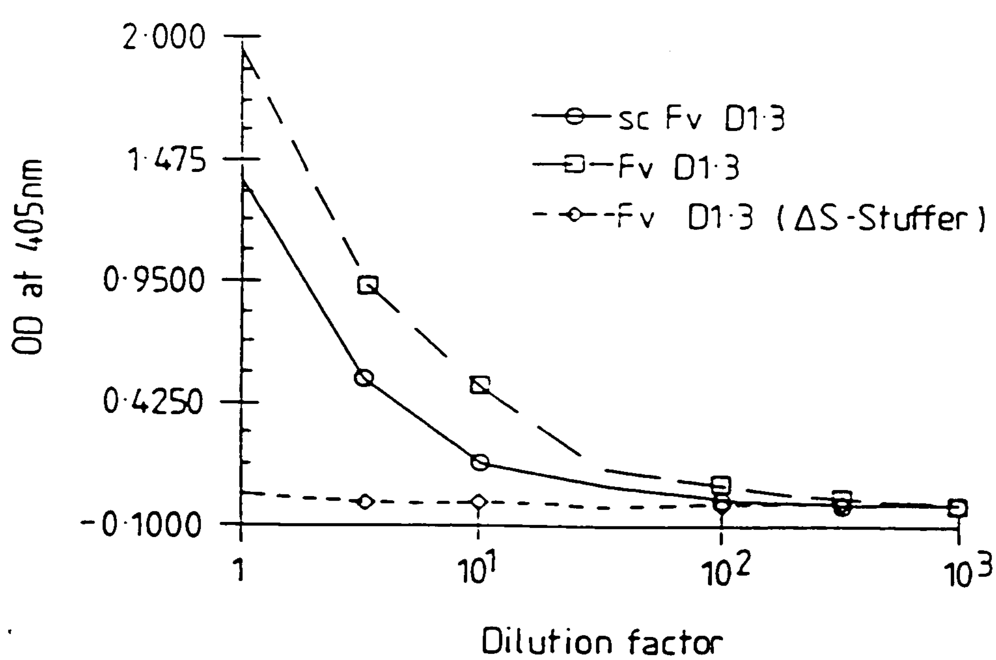

2·000
1·475
0·9500
0·4250
-0·1000
OD at 405nm
sc Fv D1·3
Fv D1·3
Fv D1·3 (ΔS-Stuffer)
1
10¹
10²
10³
Dilution factor

Fig. 47.

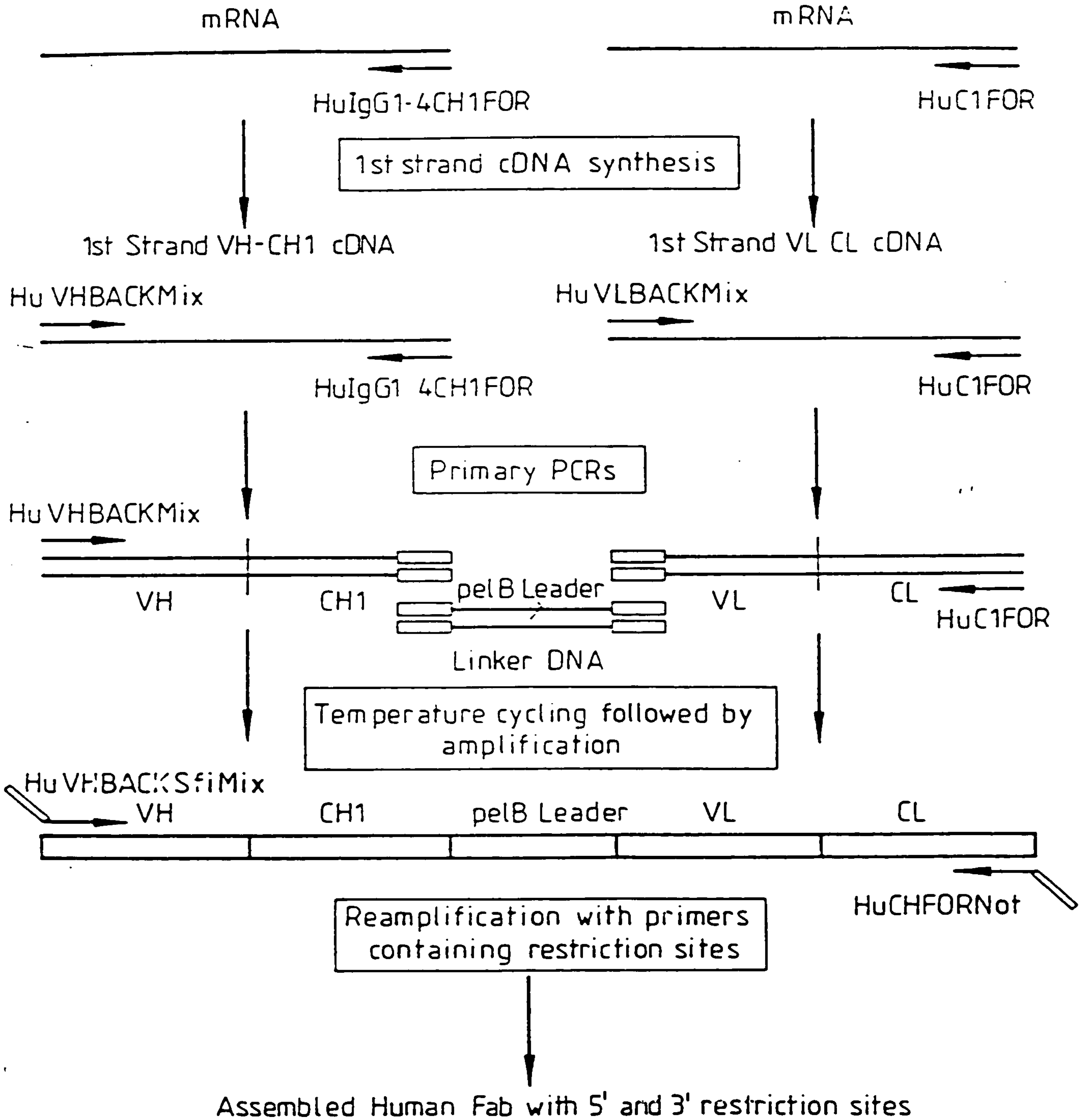
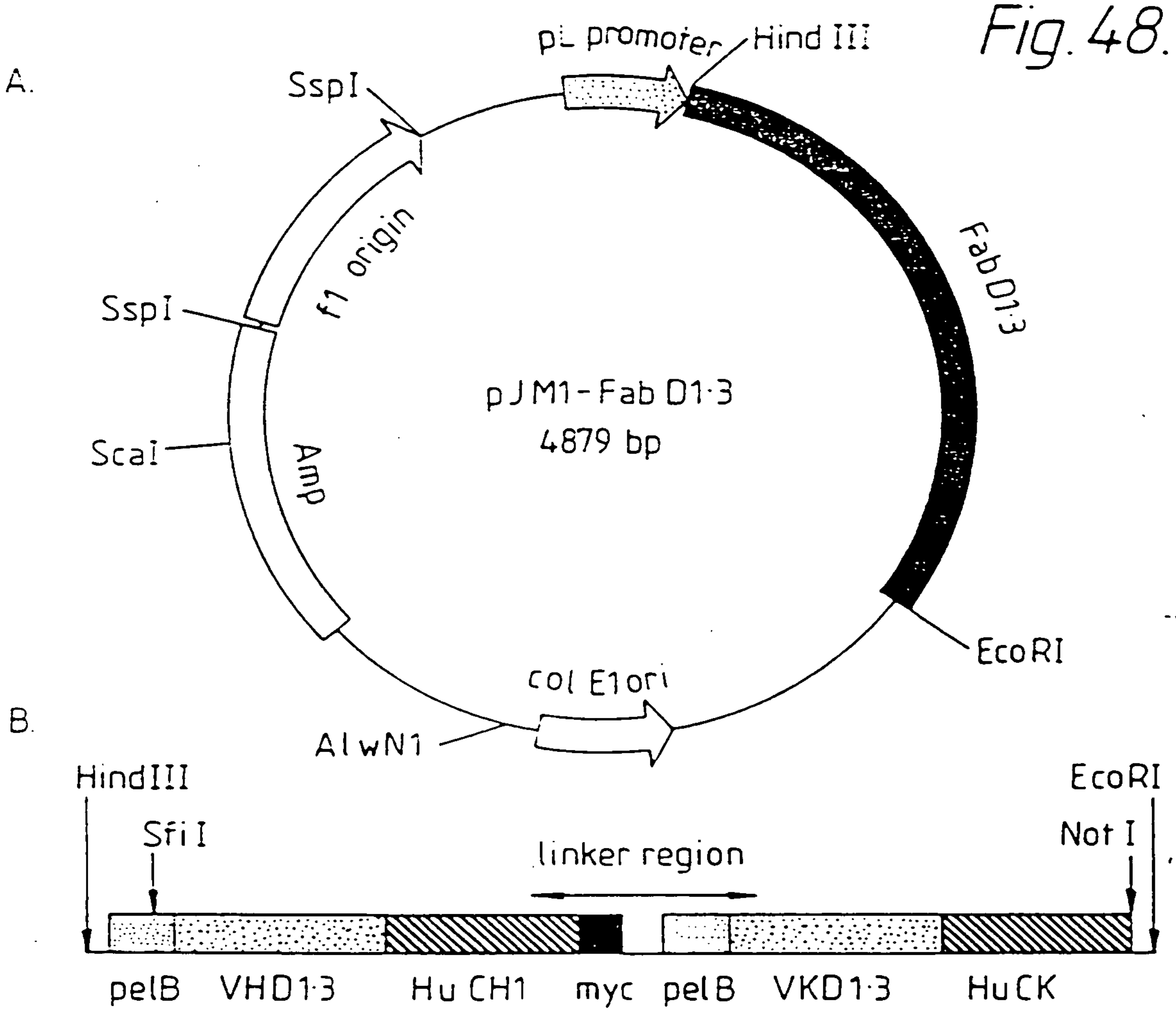

mRNA
mRNA
HuIgG1-4CH1FOR
HuC1FOR
1st strand cDNA synthesis
1st Strand VH-CH1 cDNA
1st Strand VL CL cDNA
Hu VHBACKMix
Hu VLBACKMix
HuIgG1 4CH1FOR
HuC1FOR
Primary PCRs
Hu VHBACKMix
VH
CH1
pelB Leader
VL
CL
HuC1FOR
Linker DNA
Temperature cycling followed by amplification
Hu VHBACKSfiMix
VH
CH1
pelB Leader
VL
CL
HuCHFORNot
Reamplification with primers containing restriction sites
Assembled Human Fab with 5' and 3' restriction sites

Fig. 48.

A.



C. Sequence of linker region

3' Human CH1 and hinge

K P S N T K V D K K V E P K S S T K T H T
AACCCCAGCAACACCAAGGTCGACAAGAAAGTTGAGCCCAAATCTTCAACTAAGACGCACACA

myc peptide tag

S G G E Q K L I S E E D L N * *
TCAGGAGGTGAACAGAAGCTCATCTCAGAAGAGGATCTGAATTAATAAGGGAGCTTGCATGCA

pelB leader

M K Y L L P T A A A G L
AATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGT

5' Vk

L L P A A Q P A M A D I E L T Q S P
TATTACCTGCTGCCCAACCAGCGATGGCCGACATCGAGTTCACCCAGTCTCC

Fig. 49.

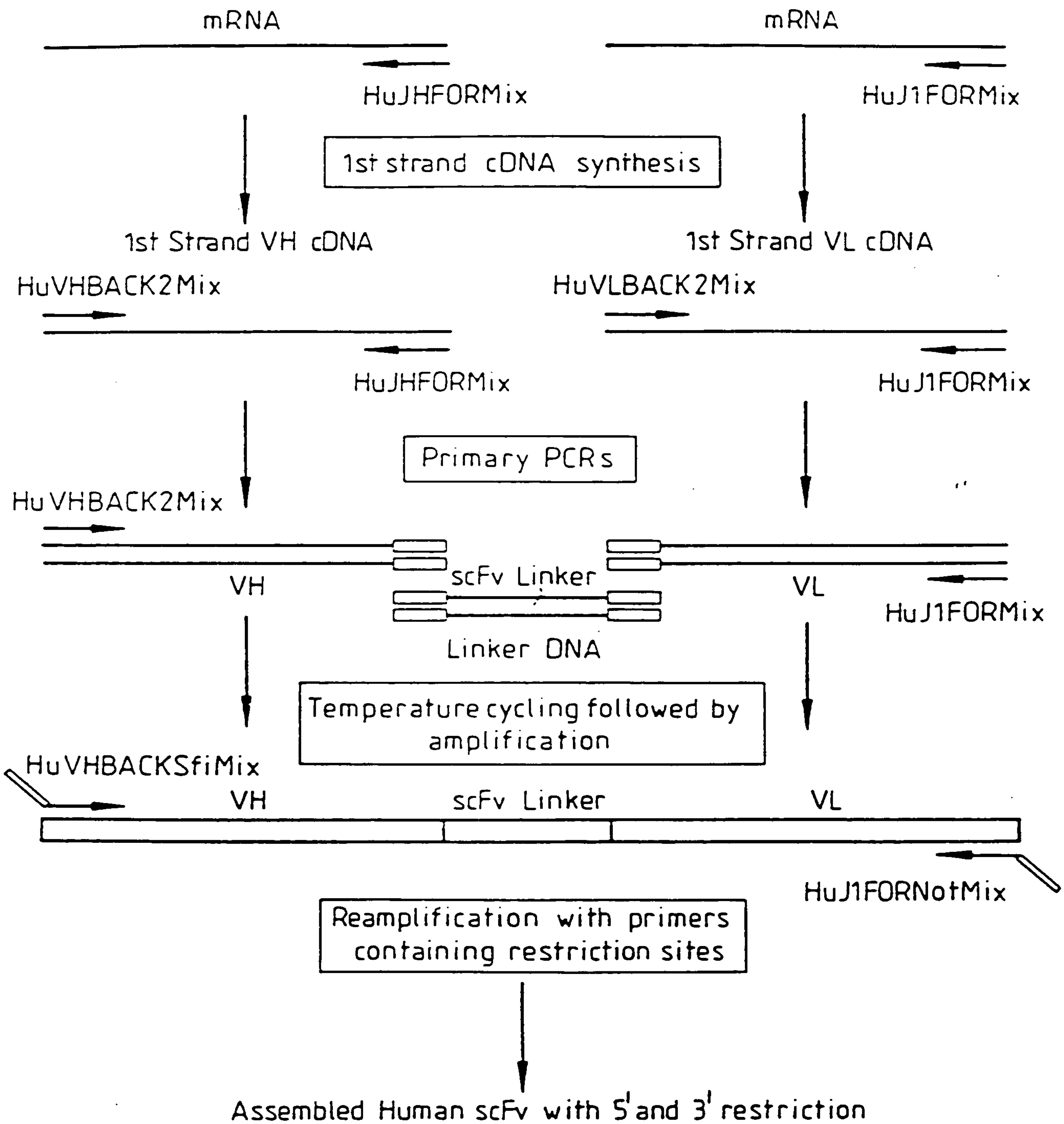

mRNA
HuJHFORMix
mRNA
HuJ1FORMix
1st strand cDNA synthesis
1st Strand VH cDNA
1st Strand VL cDNA
HuVHBACK2Mix
HuVLBACK2Mix
HuJHFORMix
HuJ1FORMix
Primary PCRs
HuVHBACK2Mix
VH
scFv Linker
VL
HuJ1FORMix
Linker DNA
Temperature cycling followed by amplification
HuVHBACKSfiMix
VH
scFv Linker
VL
HuJ1FORNotMix
Reamplification with primers containing restriction sites
Assembled Human scFv with 5' and 3' restriction

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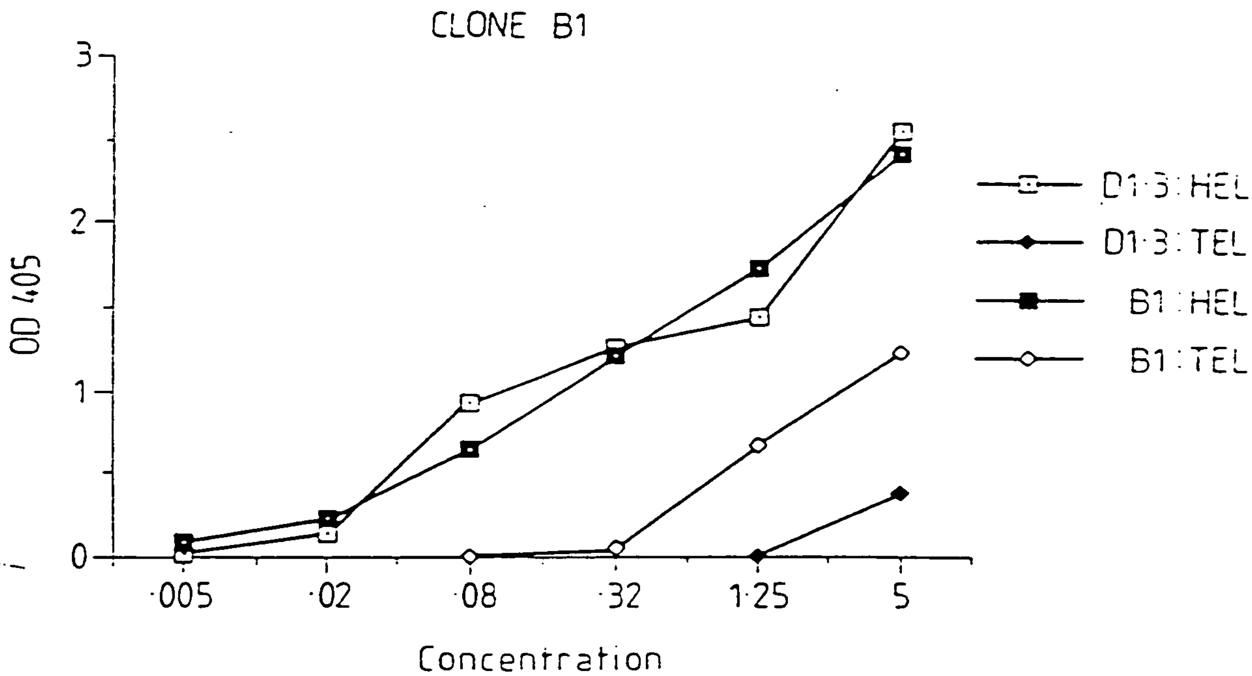
Fig. 50.


CLONE B1
OD 405
3
2
1
0
·005
·02
·08
·32
1·25
5
Concentration
D1·3:HEL
D1·3:TEL
B1:HEL
B1:TEL

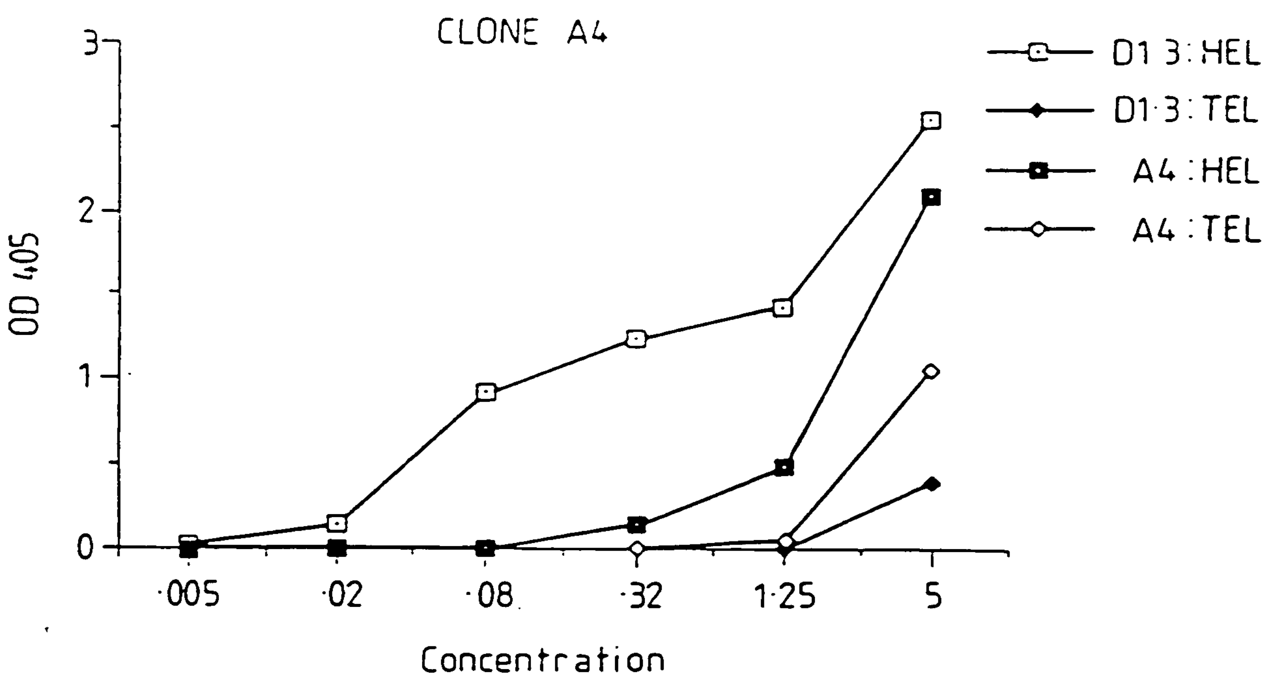

CLONE A4
OD 405
3
2
1
0
·005
·02
·08
·32
1·25
5
Concentration
D1·3:HEL
D1·3:TEL
A4:HEL
A4:TEL

Fig.51.

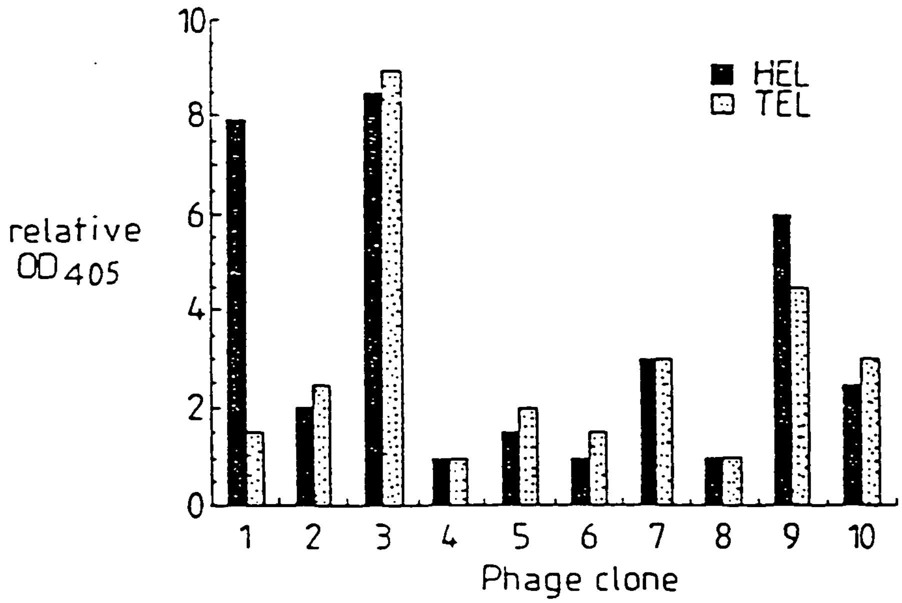

10
8
6
4
2
0
relative
OD405
HEL
TEL
1
2
3
4
5
6
7
8
9
10
Phage clone

Fig.53.

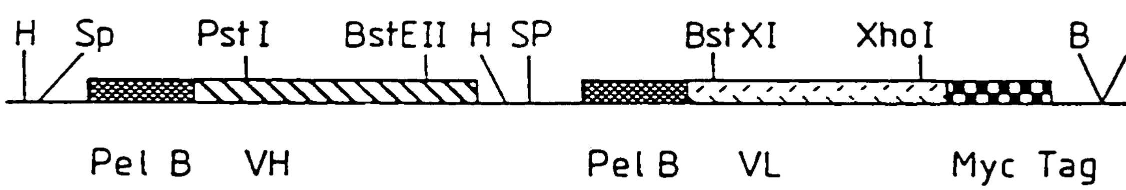

H
Sp
PstI
BstEII
H
SP
BstXI
XhoI
B
E
Pel B
VH
Pel B
VL
Myc Tag

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Fig. 52.

CDR 1 CDR 2

D1.3 DIQMTQSPASLSASVGETVTITCRASGNIHNYLA WYQQKQGKSPQLLVYYTTTLAD

M1F DIELTQSPSSLSASLGERVSLTCRASQDIGSSLN WLQQEPDGTIKRLIYATSSLDS

M21 DIELTQSPALMAASPGEKVTITCSVSSSISSSNLHWYQQKSETSPKPWIYGTSNLAS

CDR 3

D1.3 GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWSTPRTFGGGTKLEIKR

M1F GVPKRFSGSRSGSDYSLTISSLESEDFVDYYCLQYASSPWTFGGGTKLELKR

M21 GVPVRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSYPLTFGAGTKLEIKR